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Relations among Growth, Nodulation, P Efficiency and Proton Efflux for Annual Legumes

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Abstract: Although, cowpea is more tolerant to phosphorus deficiency than soybean and common bean, whether this better tolerance is related to a greater P Use Efficiency (PUE) or higher specific nodule activity is not well documented. In this study, we screened different annual legumes in the glasshouse for their genotypic diversity in PUE for Symbiotic Nitrogen Fixation (SNF) and their proton efflux from roots. After growing 4 weeks in hydro-aeroponic conditions, the plants were transferred into serum bottles and on soil bags on a Chromic Cambisol soil from France or an Acid Sandy soil from Northeast Thailand until harvested. Almost all *V. unguiculata* cultivars nodulated on the Chromic Cambisol soil, but not on the Acid Sandy soil from Thailand. In this experiment, *V. unguiculata* showed mainly a better growth under P sub-deficiency (75 $\mu\text{mol P week}^{-1}$) than P sufficiency (250 $\mu\text{mol P week}^{-1}$) and a greater PUE rather than a higher specific nodule activity. *V. unguiculata* cv. 26-73 had the lowest proton efflux among all the cultivars. However, when *V. unguiculata* was grown on the Acid Sandy soil, we noticed a significant decrease in soil pH and the P applications tended to increase soil pH. According to the results from our trials, *V. unguiculata* is the most interesting grain legume to grow as it proved to be more tolerant to phosphorus deficiency. We especially recommend using cv. 26-73, since it was responsible for a smaller H^+ efflux than the other cultivars.

Key words: Annual grain-legumes, growth, nodulation, P efficiency, proton efflux

INTRODUCTION

Phosphorus is among the most needed elements for crop production. In heavily weathered acid soils, P is generally deficient and limits the potential input of Symbiotic Nitrogen Fixation (SNF) (Waluyo *et al.*, 2004). In common bean (*Phaseolus vulgaris*), soybean (*Glycine max* (L.) Merr.), lupine (*Lupinus mutabilis*) and alfalfa (*Medicago truncatula*) P deficiency has been described (1) to reduce the number and biomass of nodules as well as their nitrogenase activity (Ribet and Drevon, 1995; Vadez *et al.*, 1996; Qiao *et al.*, 2007), (2) to increase the absorption surface and density of the roots resulting in more exploration of the soil volume (Vance, 2001) and (3) to acidify the rhizosphere by root exudates (Neumann and Römheld, 1999) and H^+ efflux (Tang *et al.*, 2001a, b, 2004).

Cowpea (*Vigna unguiculata*) is considered as being more tolerant to phosphorus deficiency than soybean and common bean (Alkama *et al.*, 2008). This better tolerance has been related to three main characters: (1) a greater P use efficiency, (2) a higher specific nodule activity and (3) different P distributions between the plant organs (Alkama *et al.*, 2008). Cowpea is thus considered as an

excellent species in respect to low P tolerance in improvement symbiotic nitrogen fixation programs. Nevertheless, this higher specific nodule activity investigation was carried out in the N-free hydroponic culture system, but not in soils where the plant can find three different sources of N (soil, fertilizer and the air). Ankomah *et al.* (1995) reported that the different tolerance to P deficiency in cowpea was related to different abilities to absorb soil P or to differences in P use efficiency to fix N_2 from atmosphere, in agreement with similar reports for other legume species like common bean, mungbean (*Vigna radiata* (L.)) and soybean (Gunawardena *et al.*, 1992, 1993; Vadez *et al.*, 1999).

However, the relation between the genotypic variation in P Use Efficiency (PUE) for the SNF and the H^+ efflux is not well documented. Moreover, one of the options for overcoming the reliance on P fertilizers for improved crop production in P-deficient soils would be the selection of low soil P-tolerant lines that could perform a greater growth together with a low proton efflux. In this study we examined annual legumes under glasshouse conditions for their genotypic diversity in P Use Efficiency (PUE) for SNF and proton efflux.

MATERIALS AND METHODS

Biological materials: Grain legumes were studied in February to May, 2006 and September, 2006 to March, 2007 at Centre international d'études supérieures en sciences agronomiques Montpellier SupAgro (INRA), France (Table 1). The seeds were first sterilized with 3% calcium hypochlorite for 15-20 min and then rinsed by 5 washings with sterile distilled water (Vincent, 1970). Thereafter, they were transferred for germination on soft agar containing 100 mL of Bergersen solution with 5 g of mannitol and 7 g of agar for 1 L of distilled water, after sterilization at 120°C for 20 min (Vincent, 1970). After germination, the inoculation was performed by immersing 4-day-old seedlings for 30 min in a suspension of *Bradyrhizobium* sp. Vigna CB756 (supplied by CSIRO, Canberra, Australia) and *Bradyrhizobium tropici* CIAT 899 (supplied by E. Martinez, UNAM Cuernavaca, Mexico) containing 10^9 bacteria mL^{-1} (Vincent, 1970). The inoculum was prepared from a rhizobia suspension preserved at 4°C on agar YEM (Yeast Extract Mannitol) medium and maintained at 28°C for 24 h prior to inoculation (Vincent, 1970).

Culture conditions in the glasshouse: The inoculated plants were transferred into three 45 L vats 0.2 m large, 0.4 m long and 0.2 m high and they were grown under hydroaeroponic conditions until flowering stage. Each vat corresponded to a P supply of 30, 75 and 250 $\mu\text{mol P/week/plant}$ in 2006 and of 30 and 75 $\mu\text{mol P week}^{-1}$ plant $^{-1}$ in 2007 in the form of KH_2PO_4 based on the work by Vadez *et al.* (1996). The composition of the nutrient solution was adjusted as CaCl_2 (1650 μM); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1000 μM); K_2SO_4 (700 μM); Fe EDDHA (8.5 μM Fe as Sequestrene®); H_3BO_3 (4 μM); $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (6 μM); $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (1 μM); $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ (1 μM); $\text{Na}_2\text{MoO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 μM) (Vadez, 1996) and this solution was changed every two weeks. The oxygenation of the solution was carried out by a permanent flow of 400 mL min^{-1} of compressed air. Urea was provided at the rate of 2 mmol plant^{-1} in the initial solution and of 1 mmol plant^{-1} until 4 weeks in order to optimize nodulation (Hernández and Drevon, 1991). Thereafter, the plants were grown in a N-free solution for annual legumes and in a 1 mmol plant^{-1} solution for perennial legumes. The whole experiment was carried out in a glasshouse with a 16/8 h day/night cycle, a temperature of 28/20°C, an additional illumination of 400 $\mu\text{mol photons/m}^2/\text{sec}^{-1}$ and a 70% relative humidity during the day. The plants were first grown in vat conditions for 4 weeks. After this pre-cultivation period, 9 plants of each cultivar were removed from the vats to be

Table 1: Used annual legumes in the experiments

Species	Cultivar	Origin
2006		
<i>Phaseolus vulgaris</i>	115	Central America
<i>Phaseolus vulgaris</i>	147	Central America
<i>Vigna unguiculata</i>	26-73	India
<i>Vigna unguiculata</i>	Danila	India
<i>Lablab purpureus</i>		India, South East Asia
2007		
<i>Vigna unguiculata</i>	26-73	India
<i>Vigna unguiculata</i>	Danila	India
<i>Vigna unguiculata</i>	305	West Africa
<i>Vigna unguiculata</i>	Kabyle	West Africa

Table 2: Chromic Cambisol soil characteristics (France)

Characteristics	Value
Clay (%)	48.60
Fine silt (%)	21.80
Coarse silt (%)	17.80
Fine sand (%)	11.60
Coarse sand (%)	3.00
pH _{H2O}	7.00
pH _{KCl}	6.10
β_1 ($\mu\text{mol OH}^{-1}$ (g soil) $^{-1}$ (pH unit) $^{-1}$)	53.73
CaCO_3 (g kg^{-1})	<0.00
Organic Matter (g kg^{-1})	24.10
CEC (cmol kg^{-1})	21.60
Ca^{2+} (cmol kg^{-1})	18.50
Na^+ (cmol kg^{-1})	0.13
Mg^{2+} (cmol kg^{-1})	1.00
K^+ (cmol kg^{-1})	0.38
P_{total} (g kg^{-1})	0.90
P_{ass} (g kg^{-1})	0.007

Chromic Cambisol soil according to FAO-UNESCO in 1989, fersiallitic soil in the old CPCS (1967) French classification

placed in 3 additional experimental sets. For each cultivar, a first group of 3 plants was transferred into serum bottles and the remaining 6 plants were grown on bags containing a given type of soil called rhizoboxes. A Chromic Cambisol soil from France was used in one set of 3 rhizoboxes and an Acid Sandy soil from Northeast Thailand was used in a second set of 3 rhizoboxes. All plants were grown until harvest.

Measurement of proton efflux in rhizobox: To evaluate the incidence of the nodulated-root on soil pH, 3 plants representing the average growth in vats of each P treatment, namely P sufficiency (250 $\mu\text{mol P week}^{-1}$), P sub-deficiency (75 $\mu\text{mol P week}^{-1}$) and P deficiency (30 $\mu\text{mol P week}^{-1}$) were transferred individually on a chromic Cambisol and an Acid Sandy soil 28 Days after Sowing (DAS) in rhizotrons (Alkama, 2008). The chromic Cambisol (CPCS, 1967) had a high cation exchange capacity, a neutral pH and a low content of Olsen-extractable phosphorus (Table 2). Whereas the Acid Sandy soil from Northeast Thailand had a low cation exchange capacity and soil pH (Table 3). It was collected

Table 3: Acid Sandy soil characteristics from Northeast (Thailand)

Characteristics	Value
Sand (%)	92.00
Silt (%)	4.00
Clay (%)	4.00
pH _{H2O}	4.90
Organic matter (%)	0.60
CEC (cmol kg ⁻¹)	1.71
K ⁺ (mg kg ⁻¹)	30.00

This data were estimated by Land Development Department, 2007

at 5-20 cm depth in Cazeveille (south of France), then sieved (<2 mm) after removing the stones and plant residues (Mengel and Kirkby, 1987). The 34 g of soil used in each rhizotron were incubated for 4 days at 20°C and a polyamide film of 30 µm mesh (Nytrel 0.2 SPN, Fyltis-U.G.B., Lyon, France) separated the soil from the roots without limiting the exchange of water and chemicals between the soil and the nodulated roots (Hinsinger and Gilkes, 1997). The rhizotrons were fixed vertically into 3 L buckets, with a filter paper used as a wick bathing in the previously described nutrient solution. The initial soil pH was measured in an aqueous suspension with a 1/5 soil/solution ratio (Alkama, 2008). At harvest, a fraction of soil of each replicate was weighed and dried at 105°C for 24 h to estimate the water content of each soil sample. The H⁺ efflux was calculated as $QH^+ = (\beta_s \Delta pH Ma) t^{-1}$, expressed in µmol plant/day with: β_s , pH buffer capacity of the soil in µmol OH⁻ g soil⁻¹ unit pH⁻¹; ΔpH , difference of pH between the pH at harvest and the initial pH before the culture; Ma , mass of soil used, in g; t , time duration of the culture, in days (Alkama, 2008). The soil pH buffer capacity was assessed by depressing or increasing soil pH by addition of a solution of H₂SO₄ or KOH (Alkama, 2008). According to the proton balance, the soil pH depends on the amount of H⁺ added or depleted from the soil solution and on the soil pH buffer capacity that depends on the content of clay and organic matter (Conyers *et al.*, 1995).

Plant and soil sampling for analysis: At flowering stage, 3 plants of each cultivar in vat conditions, serum bottles, on bags containing a Chromic Cambisol soil from France and on bags containing an Acid Sandy soil from Northeast Thailand were randomly selected and harvested. The shoot was separated from the root the cotyledonary node and both parts were weighed after drying at 70°C for 48 h (Mengel and Kirkby, 1987). The nodules were separated from the roots, counted and weighed separately according to Drevon and Hartwig (1997). The P content was determined on the shoot of each plant at harvest. 1.5 mL of 65% HNO₃ was added to 50 mg finely ground plant tissue samples in a mineralization tube, they were then fixed in the rotor

segment of an ETHOS TC digester (Terminal 320 color-touch) and heated at 180°C for 10 min. After the digestion was complete, the samples were allowed to cool. Then, the P concentration was determined by the vanado-molybdate colorimetric method by spectrophotometry (Thermo electron corporation model Genesys 10 VIS) at 460 nm. In addition, the soil samples from the rhizobox were dried at 105°C for 48 h (Mengel and Kirkby, 1987) and their pH values were measured in an aqueous suspension in a 1/5 soil/solution ratio.

Statistical data processing: Differences between means of all parameters for P treatments were determined by the Student test ($p < 0.05$), using the Statistical Analysis System (Statistic 8 User Guide version 2.0, ISBN-13: 9780072941654). To assess the P use efficiency (PUE in gDW/%P), the weights of shoot and root were divided by their P contents (Fageria *et al.*, 1997).

RESULTS

Grain legumes responses to P supply under the glasshouse conditions: The grain legume species were grown in the hydro-aeroponic culture for 4 weeks in the vats and then they were transferred to individual serum bottles or rhizotrons until they were harvested (8 weeks after sowing). Figure 1 shows the production of shoot and root biomass in the serum bottles for treatments 30 and 75 P in the case of cowpea and for treatments 75 and 250 P for cowpea, common beans and hyacinth bean (*Lablab purpureus* L.). P sub-deficiency (75P) resulted in a significantly better shoot growth (4.06, 4.06 and 2.75 g plant⁻¹, respectively) than P deficiency (30P) (2.83, 1.78 and 1.33 g plant⁻¹, respectively) for *V. unguiculata* cv. 305, Kabyle and Danila. Shoot growth of *V. unguiculata* cv. 305 and Kabyle was significantly better than shoot growth of 26-73 and Danila (4.06 and 4.06 g plant⁻¹) at P sub-deficiency (75P). By contrast, root growth was not significantly improved by P sub-deficiency (75P), even though the values for 75P were consistently slightly higher. Root growth was significantly better for *V. unguiculata* cv. 305 (1.66 g plant⁻¹) than for the other species.

P sufficiency (250P) resulted in a significant increase in shoot growth of *P. vulgaris* cv. 115 and 147 compared with P sub-deficiency (75P) (5.37 and 4.75 g plant⁻¹, respectively). By contrast, root growth of *P. vulgaris* cv. 147 was not significantly improved by P sufficiency (250P), even though the biomass for 250P was consistently slightly higher. P sufficiency (250P) did not significantly improve any biomass parameter compared to

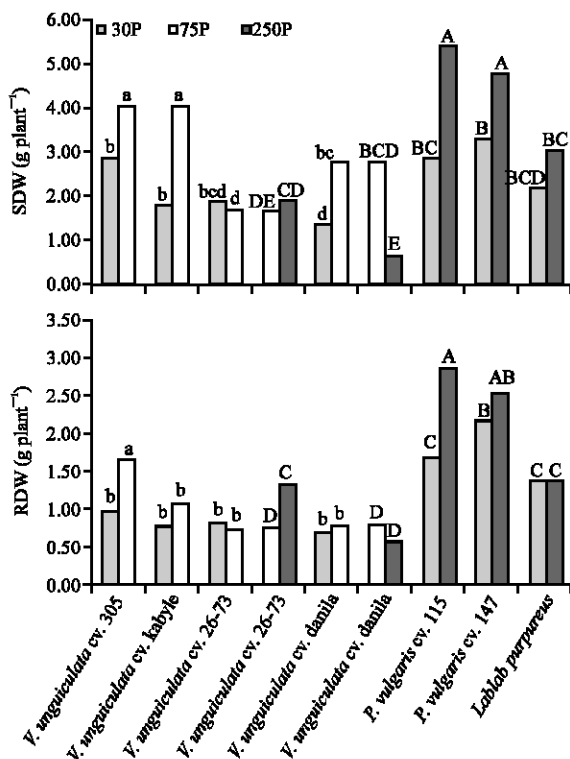


Fig. 1: Shoot and root dry weight of *Vigna unguiculata* cv. 305, Kabyle, 26-73 and Danila, *Phaseolus vulgaris* cv. 115 and 147 and *Lablab purpureus* in serum bottle conditions in hydro-aerobic culture. Data are means of 3 replicates for 30P, 75P and 250P. Data with different letters are significantly different ($p < 0.05$), with capital letters to data obtained during 1st year and small letters corresponding to data obtained during 2nd year

P sub-deficiency (75P) for *V. unguiculata* cv. Danila (0.63 and 2.75 g SDW plant⁻¹, respectively). Shoot growth was not significantly better for *V. unguiculata* cv. 26-73 at P sufficiency (250P) than at P sub-deficiency (75P). By contrast, root growth was significantly improved by P sufficiency (250P). P sufficiency (250P) did not significantly improve any biomass compared to P sub-deficiency (75P) for *V. unguiculata* cv. Danila and 26-73. *P. vulgaris* cv. 115 and 147 produced the highest shoot biomass (5.37 and 4.75 g plant⁻¹, respectively) at P sufficiency (250P) and also the highest root biomass (2.87 and 2.55 g plant⁻¹, respectively).

Figure 2 shows the production of shoot and root biomass in the rhizotrons with the Chromic Cambisol from Cazevieille (France), for the same plant species and treatments as for the serum bottles. *V. unguiculata* cv. 305 showed the highest shoot growth. P sub-deficiency

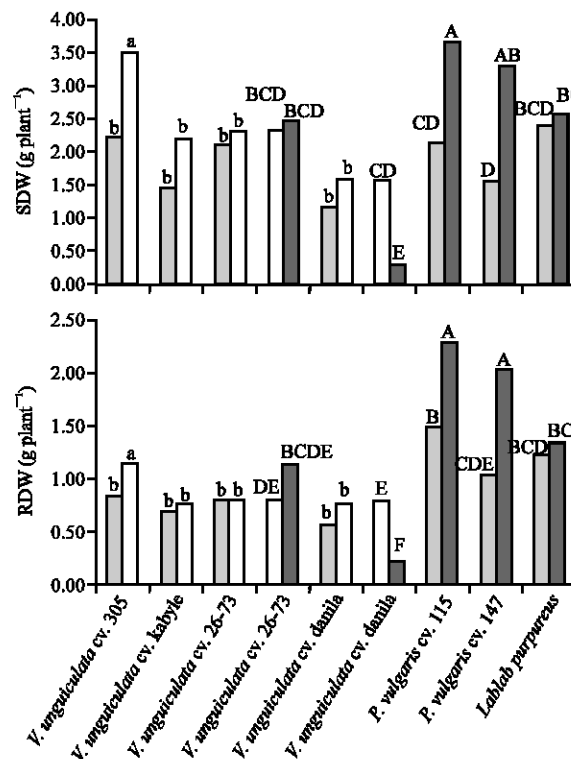


Fig. 2: Shoot and root dry weight of *Vigna unguiculata* cv. 305, Kabyle, 26-73 and Danila, *Phaseolus vulgaris* cv. 115 and 147 and *Lablab purpureus* on Chromic Cambisol soils from Cazevieille (France) in rhizotron conditions in hydro-aerobic culture. Data are means of 3 replicates for 30P, 75P and 250P. Data with different letters are significantly different ($p < 0.05$), with capital letters to data obtained during 1st year and small letters corresponding to data obtained during 2nd year

(75P) resulted in a significantly higher value (3.53 g plant⁻¹) than P deficiency (30P) (2.25 g plant⁻¹) and also for root growth. For *V. unguiculata* cv. Kabyle, 26-73 and Danila, P sub-deficiency (75P) did not produce a better growth (shoot as well as root) than P deficiency (30P).

P sufficiency (250P) resulted in a significant increase in shoot growth compared to P sub-deficiency (75P) for *P. vulgaris* cv. 115 and 147 (3.67 and 3.3 g plant⁻¹, respectively). P sufficiency (250P) did not significantly improve any biomass parameter compared to P sub-deficiency (75P) for *V. unguiculata* cv. Danila. For *V. unguiculata* cv. 26-73 at P sufficiency (250P), shoot growth was not significantly higher than P sub-deficiency (75P). *V. unguiculata* cv. Danila showed a significantly lower value of shoot growth compared to the other

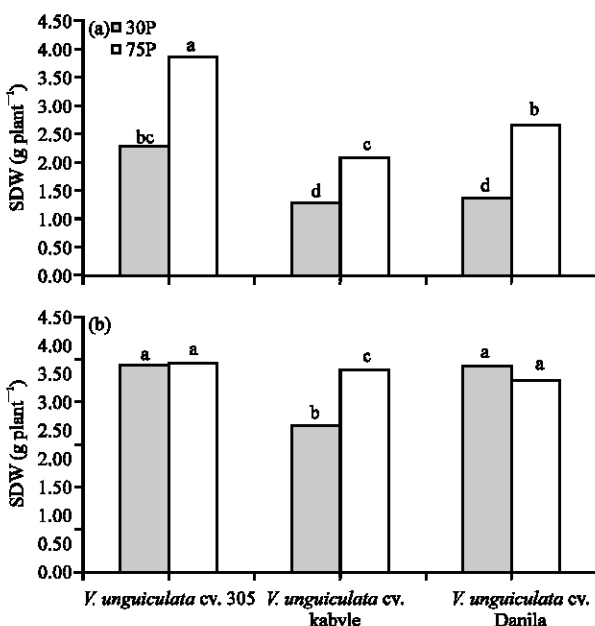


Fig. 3: Shoot (a) and root (b) dry weight of *Vigna unguiculata* cv. 305, Kabyle and Danila on Acid Sandy soil from Northeast (Thailand) in rhizotron conditions in hydro-aeroponic culture. Data are means of 3 replicates for 30P and 75P

cultivars at P sufficiency (250P). The data of root growth showed results similar to shoot growth. P sufficiency (250P) did not significantly improve any biomass parameter compared to P sub-deficiency (75P) for *V. unguiculata* cv. Danila and 26-73.

The shoot and root biomass of the three cowpea cultivars grown in rhizotrons on the Acid Sandy soil from Northeast Thailand at P deficiency (30P) and P sub-deficiency (75P) are presented in Fig. 3. P sub-deficiency (75P) resulted in a significantly higher value (3.86, 2.65 and 2.09 g plant⁻¹, respectively) than P deficiency (30P) (2.25, 1.35 and 1.28 g plant⁻¹, respectively) (Fig. 3a). By contrast, root growth of *V. unguiculata* cv. 305 and Danila was not significantly improved by P sub-deficiency (75P) (Fig. 3b). The data for root growth showed similar results as shoot growth for *V. unguiculata* cv. Kabyle. *V. unguiculata* cv. 305 gave the highest shoot growth (3.86 g plant⁻¹) at P sub-deficiency.

Nodulation: Even though the seeds of *Vigna* sp. were inoculated with *Bradyrhizobium* sp. *Vigna* CB756, the symbiosis did not develop properly on the *Vigna* in hydro-aeropony. P sub-deficiency (75P) did not result in an increase in the nodule weight or the number of nodule of the cowpeas, even though the value for P sub-deficiency (75P) was consistently slightly higher (data not

shown). Whereas, *P. vulgaris* cv. 115 gave a significantly higher weight of nodule (0.5 g plant⁻¹ against 0.02 g plant⁻¹) with P sufficiency (250P) than with P sub-deficiency (75P). The higher weight of *P. vulgaris* cv. 147 was the results of an increase in the number of nodules (49 nodules plant⁻¹). Also, P sufficiency (250P) did not significantly improve the weight of the nodules nor the number of nodules compared to P sub-deficiency (75P) for *V. unguiculata* cv. Danila and 26-73. However, the feeble development of nodulation on the *Vigna* species makes the comparisons difficult.

The weight of the nodules for the *Vigna* sp. in the rhizotrons on the Chromic Cambisol soil from Cazeville (France) was low, just slightly better than in hydro-aeropony. For *V. unguiculata* cv. 305 P sub-deficiency (75P) resulted in a significant increase in the weight of the nodules, but the increase in the number of nodules was not significant (data not shown). P sub-deficiency (75P) did not result in an increase in the weight of the nodules nor the number of nodules for the other cultivars, even though the value for P sub-deficiency (75P) was consistently slightly higher (except the number of nodules for *V. unguiculata* cv. Kabyle that was slightly lower).

P. vulgaris cv. 115 showed a significant increase in nodule weight between P sub-deficiency (75P) and P sufficiency (250P) with respective values of 0.65 and 1 g plant⁻¹. *P. vulgaris* cv. 147 showed a significant increase in nodule weight between P sub-deficiency (75P) and P sufficiency (250P) with respective values of 0.33 and 1.01 g plant⁻¹. This increase resulted from a significantly higher number of nodules per plant (19.67 and 256 nodules plant⁻¹ for *P. vulgaris* cv. 115 and 12.33 and 254 nodules plant⁻¹ for *P. vulgaris* cv. 147). By contrast, the weight of the nodules and the number of nodules of *V. unguiculata* cv. Danila and 26-73 were not significantly increased by P sufficiency (250P).

No nodulation was observed for *V. unguiculata* cv. 305, Kabyle and Danila at both P deficiency (30P) and P sub-deficiency (75P) in the rhizotrons on the Acid Sandy soil from Northeast Thailand (data not shown).

P use efficiency: Figure 4 shows the PUE in shoot and total PUE plant⁻¹ for the cowpea species *V. unguiculata* cv. 305, Kabyle, 26-73 and Danila grown in serum bottles. P sub-deficiency (75P) did not significantly improve PUE compared to P deficiency (30P). *V. unguiculata* cv. 305 gave a significantly higher value of PUE in shoot (Fig. 4a) and total plant (Fig. 4b) (39.76 gSDW/%P and 52.08 gDW/%P, respectively) than the other cultivars at P deficiency (30P).

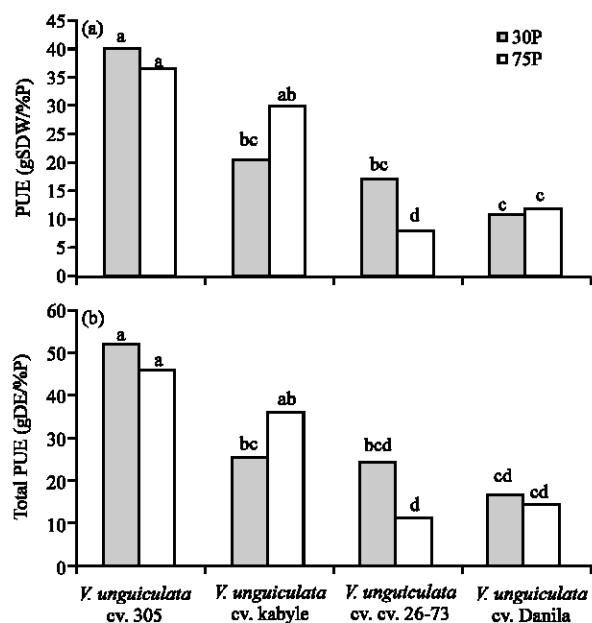


Fig. 4: PUE in shoot biomass (gSDW/%P) (a) and total PUE (gDW/%P) (b) of *Vigna unguiculata* cv. 305, Kabyle, 26-73 and Danila in serum bottle conditions in hydro-aeroponic culture. Data are means of 3 replicates for 30 and 75P

In rhizotron on the Chromic Cambisol soil from Cazevieille (France) P deficiency (30P) resulted in a significantly higher value (24.01 gSDW/%P and 27.44 gDW/%P) than P sub-deficiency (75P) (7.94 gSDW/%P and 11.72 gDW/%P) for *V. unguiculata* cv. 26-73. For *V. unguiculata* cv. Danila there was no difference between the two treatments (Fig. 5a, b).

P deficiency (30P) resulted in significantly higher values of PUE (35.71 gSDW/%P and 37.64 gDW/%P, respectively) (Fig. 6a, b) than P sub-deficiency (75P) for *V. unguiculata* cv. 305 in rhizotron on an Acid Sandy soil from Northeast Thailand. PUE was not significantly improved by P deficiency (30P) for *V. unguiculata* cv. Kabyle, even though the value for P deficiency (30P) was slightly higher. By contrast, P sub-deficiency (75P) resulted in a significantly higher value (16.15 gSDW/%P) (Fig. 6a). However, PUE was not significantly improved by P sub-deficiency (75P) in the total plant (Fig. 6b), even though the value for P sub-deficiency (75P) was slightly higher. *V. unguiculata* cv. 305 gave a significantly higher value of PUE in the shoot and in total plant than the other cultivars.

Proton efflux: The nutrient solution pH did not change during the course of the experiment for both 30P and 75P in serum bottles (data not shown). However, the pH was

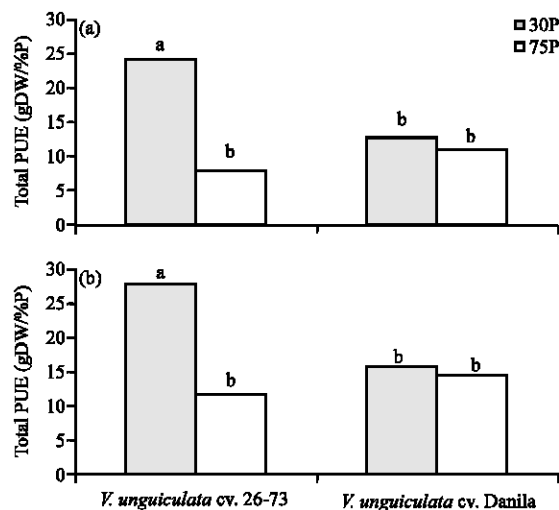


Fig. 5: PUE in shoot biomass (gSDW/%P) (A) and total PUE (gDW/%P) (B) of *Vigna unguiculata* cv. 26-73 and Danila on Chromic Cambisol soils from Cazevieille (France) in rhizotron conditions in hydro-aeroponic culture. Data are means of 3 replicates for 30 and 75P

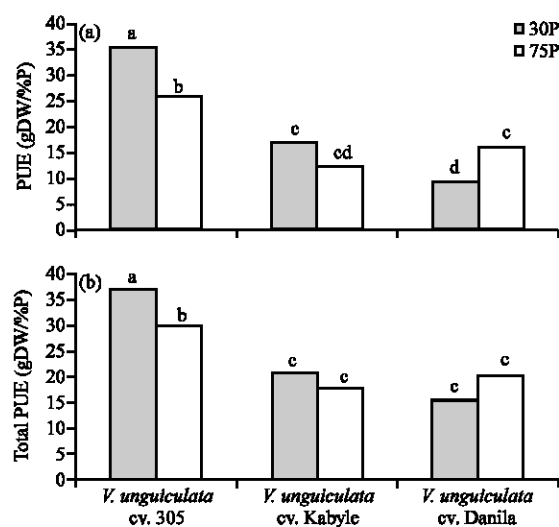


Fig. 6: PUE in shoot biomass (gSDW/%P) (a) and total PUE (gDW/%P) (b) of *Vigna unguiculata* cv. 305, Kabyle and Danila on Acid Sandy soil from Northeast Thailand in rhizotron conditions in hydro-aeroponic culture. Data are means of 3 replicates for 30 and 75P

higher for *V. unguiculata* cv. 26-73 than for other cultivars. This may be due to the fact that this cultivar absorbed more PO_4^{2-} or HPO_4^- than the other cultivars, triggering a lower release of H^+ in the nutrient

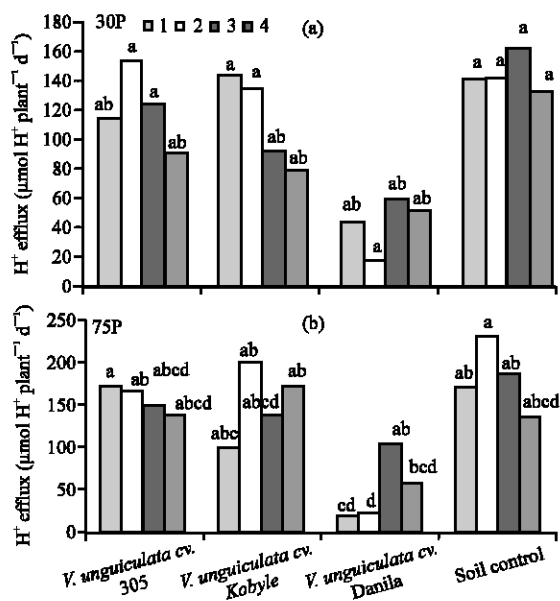


Fig. 7: Proton efflux of *Vigna unguiculata* cv. 305, Kabyle, 26-73 and Danila at 1-4 week after transferred in serum bottle conditions in hydro-aeroponic culture. Data are means of 3 replicates for 30P and 75P

solution for ion balance. The H⁺ efflux in the solution (µmol H⁺/plant/day) was deduced from the quantity of KOH added to maintain the pH. Figure 7a and b show the H⁺ efflux for *V. unguiculata* cv. 305, 26-73 Kabyle and Danila. The H⁺ efflux did not change significantly during the course of the experiment, except for *V. unguiculata* cv. 26-73 for 75P. Three weeks after the plants were transferred in serum bottles the H⁺ efflux significantly increased for this cultivar, compared to the second week (102.22 and 20.44 µmol H⁺ plant/day). *V. unguiculata* cv. 26-73 gave a smaller H⁺ efflux than the other cultivars.

The soil pH (7 units) was not significantly changed by P sub-deficiency (75P) for the cowpea species and P sufficiency (250P) for cowpea, common beans and *Lablab purpureus* in the rhizotrons on the Chromic Cambisol soil from Cazeville (France). However, the higher doses of P application tended to increase slightly the soil pH, even for the control without plant.

Data of the rhizotrons on the Acid Sandy soil from Northeast Thailand also show that, the soil pH at the end of the experiment for *V. unguiculata* cv. 305, Kabyle and Danila were not significantly modified by the application of 75P, compared to 30P, even for the control without plant. Growing cowpea significantly decreased soil pH (Fig. 8).

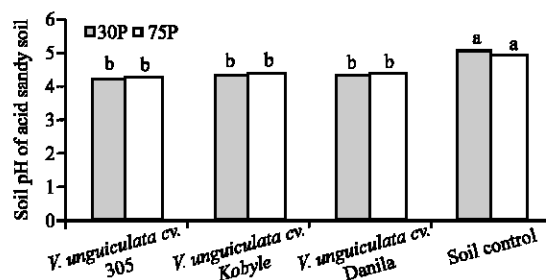


Fig. 8: Soil pH of *Vigna unguiculata* cv. 305, Kabyle, Danila and soil control on Acid Sandy soil from Northeast Thailand in rhizotron conditions in hydro-aeroponic culture. Data are means of 3 replicates for 30P and 75P

DISCUSSION

Our screening of the growth ability of *V. unguiculata*, *P. vulgaris* and *L. purpureus* on soil bags using the Chromic Cambisol soil from Cazeville (France) and the Acid Sandy Soils from Northeast Thailand in rhizotrons revealed that *V. unguiculata* cv. 305 is an interesting grain legume to grow because of its high tolerance to phosphorus deficiency which P sub-deficiency (75P) resulted in a better shoot growth (4.06, 3.53 and 3.86 gSDW plant⁻¹, respectively) (Fig. 1-3). Since, on Acid Sandy soils, *V. unguiculata* cv. 305 grew without nodulation, this better tolerance was thus mainly related to a higher P use efficiency rather than to a higher specific nodule activity and different P distributions between the plant organs. However, P deficiency reduces the number and biomass of nodule as well as their nitrogenous activity (Ribet and Drevon, 1995; Vadez *et al.*, 1996; Qiao *et al.*, 2007). This conclusion was also confirmed for *V. unguiculata* cv. 305 PUE under 30 µmol P week⁻¹ in serum bottles and in Acid Sandy Soils from Northeast Thailand reaching, respectively 40 g SDW/%P and 36 g SDW/%P (Fig. 4a, 6a). In addition our results indicate the same trend as Alkama *et al.* (2008) suggesting that cowpea is more tolerant to phosphorus deficiency than soybean and common bean. Also, similarly with the report of Ankomah *et al.* (1995) in which the different tolerance to P deficiency in cowpea was related to different abilities to absorb soil P or to differences in P use efficiency. Moreover, the above-ground biomass of *V. unguiculata* cv. 26-73, Danila and *L. purpureus* was not increased under 250 µmol P week⁻¹ compared to 75 µmol P week⁻¹, indicating that the critical P supply for these lines was less than 250 µmol P week⁻¹. From these results, we assumed that *V. unguiculata* cv. 305 may respond in a similar way under the 250 µmol P week⁻¹ as *V. unguiculata* cv. 26-73 and Danila.

The comparison of the nodulation of cowpea cultivars between the Chromic Cambisol soil from Cazeville (France) and the Acid Sandy soils from Northeast Thailand revealed that almost all cowpea cultivars nodulated on the Chromic Cambisol soil from Cazeville (France), which has a neutral soil pH, but did not nodulate on the Acid Sandy soil from Northeast Thailand, which soil pH is around 4. These observations are consistent with those of Poehlman (1991), who pointed out that the rhizobial activity and the number of nodules decreased when the soil pH was lower than 4, concluding that the suitable soil pH for rhizobial activity was 6.5. By contrast, *L. purpureus* did not nodulate in serum bottle nor on the Chromic Cambisol soil from Cazeville (France). Although we used the strain of *Bradyrhizobium* sp. Vigna CB 756, which is the most suitable to grow *L. purpureus* (Norris, 1967), the inoculant may not have been effective to induce nodulation. In the bottle conditions of our experiment, *Bradyrhizobium* sp. Vigna CB 756 may also have been affected by the transfer from the vat conditions to the Chromic Cambisol soil from Cazeville (France) and not have been present in the Chromic Cambisol soil from Cazeville (France) for a new rhizobial infection.

Rhizosphere acidification by legume roots is linked to the release of protons following excess uptake of cations over anions during N₂ fixation (Israel and Jackson, 1982; Haynes, 1983; Lui *et al.*, 1989). The H⁺ efflux in the solution ($\mu\text{mol H}^+/\text{plant}/\text{day}$) was deduced from the quantity of KOH added to maintain the pH of the nutrient solution. *V. unguiculata* cv. 26-73 gave a smaller H⁺ efflux ($20.44 \mu\text{mol H}^+/\text{plant}/\text{day}$) than the other cultivars. This lower H⁺ efflux of *V. unguiculata* cv. 26-73 compared to other cultivars is substantiated by the low P accumulation ($2.42 \text{ mg plant}^{-1}$ in SDW) of *V. unguiculata* cv. 26-73 in serum bottles. However, low soil P-tolerant lines had a greater growth with a high proton efflux. This may explain that high P accumulation will release more H⁺ efflux. In this experiment, the soil pH was not significantly modified by the application of $75 \mu\text{mol P week}^{-1}$, compared to $30 \mu\text{mol P week}^{-1}$, even for the control without plant. In the acid sandy soils, the soil pH decreased from 5 to 4 because of the growing cowpea (Fig. 8). Therefore, *V. unguiculata* cv. 26-73 also appeared interesting to grow in Northeast Thailand field to limit the acidification of soils already acidic. By contrast, the other cultivars had a higher H⁺ efflux than *V. unguiculata* cv. 26-73.

CONCLUSION

After analyzing most of the plant parameters (growth, nodulation, P accumulation and H⁺ efflux) under the serum

bottle and rhizotrons conditions, *V. unguiculata* cv. 305 appeared to be the most interesting grain legume to grow as it proved to be more tolerant to phosphorus deficiency. Its high growth at $75 \mu\text{mol P week}^{-1}$ was related to a greater P use efficiency. In addition, *V. unguiculata* cv. 26-73 also appeared interesting to grow in Northeast Thailand, since it is responsible for a smaller H⁺ efflux than the other cultivars.

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REFERENCES

- Alkama, N., E.B.B. Bolou, H. Vailhe, L. Roger, S.M. Ounane and J.J. Drevon, 2008. Genotypic variability in P use efficiency for symbiotic nitrogen fixation is associated with variation of proton efflux in cowpea rhizosphere. *Soil Biol. Biochem.* (In Press).
- Ankomah, A.B., F. Zapata, S.K.A. Danso and H. Axmann, 1995. Cowpea varieties differences in uptake of phosphorus from Gafsa phosphate rock in a low-P Ultisol. *Fert. Res.*, 41: 219-225.
- CPCS, 1967. Classification des sols. ENSA Grignon, multigr, pp: 87.
- Conyers, M.K., N.C. Uren and K.R. Helyar, 1995. Causes of changes in pH in acidic mineral soils. *Soil Biol. Biochem.*, 27: 1383-1392.
- Drevon, J.J. and U.A. Hartwig, 1997. Phosphorous deficiency increases the argon induced decline of nodule nitrogenase activity in soybean and alfalfa. *Planta*, 201: 463-469.
- Fageria, N.K., V.C. Baligar and C.A. Jones, 1997. Growth and Mineral Nutrition of Field Crops. Marcel Dekker Inc., New York, ISBN: 9780824700898
- Gunawardena, S.F.B.N., S.K.A. Danson and F. Zapata, 1992. Phosphorus requirement and nitrogen accumulation by Three mungbean (*Vigna radiata* (L.) welzek) cultivars. *Plant Soil.*, 147: 267-274.
- Gunawardena, S.F.B.N., S.K.A. Danson and F. Zapata, 1993. Phosphorus requirement and source of nitrogen in three soybean (*Glycine max*) genotypes, Bragg, nts 382 and Chippewa. *Plant. Soil.*, 151: 1-9.
- Haynes, R.J., 1983. Soil acidification induced by leguminous crops. *Grass. Forage Sci.*, 38: 1-11.

- Hernández, G. and J.J. Drevon, 1991. Influence of oxygen and acetylene during *in situ* open-flow assay of nitrogenase activity (C₂H₂ reduction) in *Phaseolus vulgaris* root nodules. J. Plant. Physiol., 38: 587-590.
- Hinsinger, P. and R.J. Gilkes, 1997. Dissolution of phosphate rock in the rhizosphere of five plant species grown in acid, P-fixing mineral substrate. Geoderma., 75: 231-249.
- Israel, D.W. and W.A. Jackson, 1982. Ion balance, uptake and transport processes in N₂ fixing and nitrate and urea dependent soybean plants. Plant Physiol., 69: 171-178.
- Lui, W.C., L.J. Lund and A.L. Page, 1989. Acidity produced by leguminous plants through symbiotic dinitrogen fixation. J. Exp. Qual., 18: 529-534.
- Mengel, K. and E.A. Kirkby, 1987. Principles of Plant Nutrition. 4th Edn., International Potash Institute, Bern, Switzerland, ISBN: 3906535037.
- Neumann, G. and V. Römheld, 1999. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant Soil., 211: 121-130.
- Norris, D.O., 1967. The intelligent use of inoculants and lime pelleting for tropical legumes. Trop. Grasslds., 1: 107-121.
- Poehlman, J.M., 1991. The mungbeans. Mohan Primlani for Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, <http://indianmedicine.eldoc.ub.rug.nl/root/P/86790/?pFullItemRecord=ON>.
- Qiao, Y.F., C. Tang, X.Z. Han and S.J. Miao, 2007. Phosphorus deficiency delays the onset of nodule function in soybean (*Glycine max* Murr.). J. Plant Nutrit., 30: 1341-1353.
- Ribet, J. and J.J. Drevon, 1995. Phosphorus deficiency increases the acetylene-induced decline in nitrogenase activity in soybean (*Glycine max* (L.) Merr.). J. Exp. Bot., 46: 1479-1486.
- Tang, C., P. Hinsinger, B. Jaillard, Z. Rengel and J. Drevon, 2001a. Effect of phosphorus deficiency on the growth, symbiotic N₂ fixation and proton release by two bean (*Phaseolus vulgaris*) genotypes. Agronomy, 21: 683-689.
- Tang, C., P. Hinsinger, J.J. Drevon and B. Jaillard, 2001b. Phosphorus deficiency impairs early nodule functioning and enhances proton release in roots of *Medicago truncatula* L. Ann. Bot., 88: 131-138.
- Tang, C., J.J. Drevon, B. Jaillard, G. Souche and P. Hinsinger, 2004. Proton release of two genotypes of bean (*Phaseolus vulgaris* L.) as affected by N nutrition and P deficiency. Plant Soil., 260: 59-68.
- Vadez, V., F. Rodier, H. Payre and J.J. Drevon, 1996. Nodule permeability to O₂ and nitrogenase linked respiration in bean landraces varying in the tolerance of N₂ fixation to P deficiency. Plant Physiol. Biochem., 34: 871-878.
- Vadez, V., J.H. Lasso, D.P. Beck and J.J. Drevon, 1999. Variability of N₂ fixation in common bean (*Phaseolus vulgaris* L.) under P deficiency is related to Puse efficiency. Euphytica., 106: 231-242.
- Vance, C., 2001. Symbiotic nitrogen fixation and phosphorus acquisition, plant nutrition in world of declining renewable resources. Plant Physiol., 127: 390-397.
- Vincent, J.M., 1970. A Manual for the Practical Study of Root-Nodule Bacteria. IBP Handbook No. 15, Blackwell Scientific Publications Ltd., Oxford, UK., ISBN: 0632064102.
- Waluyo, S.H., T.A. Lie and L. 't Mannetje, 2004. Effect of phosphate on nodule primordia of soybean (*Glycine max* Merrill) in acid soils in rhizotron experiments. in acid soil in rhizotron experiments. Indonesian J. Agric Sci., 5: 37-44.