International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(2): 391-402 June 2014
DOI: 10.5958/2230-732X.2014.00260.5

Soil Science

Phosphorus Availability and Proton Efflux of Nodulated-root Varies among Common-bean Genotypes (*Phaseolus vulgaris*) in Rhizobox

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Paper No. 224 Received: January 03, 2014 Accepted: February 28, 2014 Published: May 29, 2014

Abstract

In this study we compared between six RILs of (Phaseolus vulgaris L.) in growth, nodulation, phosphorus use efficiency for N_2 fixation and proton release in response to two levels of P in nutrient solution. For plants grown at 250 and 75 ìmol P pl^{-1} $week^{-1}$ for 42 days. For this propose Glass house experiment in rhizobox was conducted to evaluate six common bean genotypes for their growth behavior at two levels of soil phosphorus, deficient (75 µmol $week^{-1}$ $plant^{-1}$) and sufficient (250 µmol $week^{-1}$ $plant^{-1}$) using 24g soil per rhizobox. Genotypes showed differential behavior at both P levels for all parameters. Shoot biomass and nodule biomass were observed higher in sufficient P than in deficient P. Difference for phosphorus utilization efficiency and phosphorus uptake were also observed where RILs 34,75 and 147 were the higher efficient in use and uptake of P under sufficient P than deficient P. and also greater in H^+ efflux for these RILs under sufficient than deficient P. It concluded that these RILs have the possibility to enrich the poor phosphorus soils by available P and improve the soil fertility.

Highlights

- Increasing P acquisition by root exudation and by H+ efflux to release P to the plant
- Enhancing P utilization by internal mechanisms associated with efficient use of absorbed P

Keywords: Bio-availability, Genotypes, N_2 fixation, Soil, Rhizobox, Phosphorus use efficiency

Introduction

Phosphorus deficiency is a common nutritional problem affecting crop production globally (Fairhust *et al.*, 1999). Billions of hectares worldwide (> 30% of world's arable land) are considered to contain too little P to sustain adequate plant growth (Vance *et al.*, 2003). Despite having rich total P contents, the available P contents in even most fertile soils are too low to meet most plants' demands due to precipitation with Ca in alkaline soils (Rahmatullah *et. al.*, 1994) and with Al and Fe oxides in acid soils (Plaxton & Carswell, 1999; Raghothama, 1999). Low use efficiency of applied P (15-20%) in soils makes P fertilization not only uneconomical but also environmentally unsafe practice

(Vance *et al.*, 2003). The ever rising prices of P fertilizers in the world market besides increasing concern of environmental degradation (Vance *et al.*, 2003) call for multidimensional solutions to tackle the problem, instead of relying upon conventionally available high input approaches.Nature has bestowed higher plants with a number of morphological and physiological strategies to explore P under limiting conditions (Hinsinger, 1998; Vance *et al.*, 2003). The adaptive mechanisms include decreased growth rate, increased growth per unit of P uptake, remobilization of internal P (Plaxton & Carswell, 1999), increased production and secretion of phosphatases, exudation of organic acids (Raghothama, 1999; Hinsinger *et al.*, 2003,Rakshit and Bhadoria,2007,Rakshit and Bhadoria,2010) and increased root surface area due to more root growth, and changes in root morphology (Lynch & Brown, 2001; Vance *et al.*, 2003).Recently, (Zhou *et al.*, 2009) showed that faba bean can release significant amounts of proton in comparison with soybean and maize. (Li *et al*., 2008) also noted a higher acid phosphatase activity in the rhizosphere of two rice genotypes: Zhongbu 51 and Pembe.

P deficiency considers one of the major limiting factor for the production of bean (*Phaseolus vulgaris* L.), especially for plants relying on symbiotic N2 fixation (Vadez *et al*., 1999). In that respect, bean genotypes differing in their ability to fix N2 and in P-use efficiency under P stress have been identified (Pereira and Bliss, 1989; Yan *et al*., 1995; Vadez *et al*., 1999). (Kouas *et al*., 2008), we found that in common bean grown under symbiotic nitrogen fixation, the proton efflux by nodulated roots was 25% to 50% higher in BAT 477 than in CocoT under optimal to P-limiting supplies. (Ma *et al*., 2009) reported that transgenic expression of a purple acid phosphatase gene in white clover plants increased their abilities of utilizing organic phosphorus in response to P deficiency. (Sungthonwises k *et al.*, 2009). 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.

As shown for other plant species, such genotypic difference in P acquisition efficiency may also be related to their ability to alter rhizosphere conditions(Singh *et al*.,2003) that are known to influence the bioavailability of soil P, via the release of protons, organic anions or phosphatase-like enzymes (Hinsinger, 2001; Raghothama, 1999). Proton release is known to be largely influenced by the nitrogen (N) nutrition of the plant, as related to the balance of cations over anions taken up and, hence, to the source of N taken up. Selon of (Nicolas, D. 2009) The effect of changes of pH at the availability of P allow to predict low availability of phosphorus absorbed in the plant. Indeed, N can be positively charged (ammonium) and favor large proton release, negatively charged (nitrate) and favor hydroxyl release, or uncharged in the case of legumes reliant on N2 fixation (Raven and Smith, 1976; Römheld, 1986; Hinsinger *et al*., 2003). In the latter case, proton release and, hence rhizosphere acidification is expected to occur, although to a lesser extent than when N is supplied as ammonium. Increased rhizosphere acidification as a response to P deficiency has been shown for many species including nitrate-fed legumes, (Le Bot *et al*., 1990; Neumann and Römheld, 1999; Hinsinger, 2001; Hinsinger *et al*., 2003). Recently, (Pan *et al*., 2008) reported that in soybean, the P-efficient genotypes were characterized by high root-to-shoot DW ratio, together with high root length and surface area and P uptake, under P deficiency.

Plant species and even cultivars within species differ greatly in one or more of these adaptations (Gill *et al.*, 2002; Aziz *et al*., 2005) and hence, differ in P acquisition and/or utilization. Plants that are efficient in absorption and utilization of nutrients greatly enhance fertilizer use efficiency, reducing cost of inputs, and preventing losses of nutrients. These genetic differences can be exploited to develop crop cultivars efficient in P acquisition and/or utilization. (Fageria and Baligar, 1997; Kosar *et al.*, 2002). In case of P, this strategy will not only help in categorizing the existing genetic material for increased P efficiency but will also provide database for future breeding ventures (Gill *et al.*, 2004). In common bean, soybean, lupin and alfalfa, P deficiency has been shown to reduce the number and biomass of nodules 5302 Afr. J. Biotechnol.as well as their nitrogenase activity (Qiao *et al*., 2007).

In comparison, similar effect has been seldom reported for legumes relying on N2 fixation (Tang *et al*., 2001a and b). A previous work (Tang *et al*., 2001a) with N2-fixing bean showed that little difference in proton release was found between P-sufficient and P-deficient plants, and between BAT477 and DOR364 genotypes. However, this was deduced from bulk measurements at the whole plant level, whereas some other previous works have shown that the enhanced release of protons under P deficiency can be located to restricted root zones such as behind the

root apices (Hinsinger *et al*., 2003). This enhanced acidification of the rhizosphere might be related to a decrease in nitrate uptake in response to P deficiency, and to a consequent increase in the excess of cation over anion uptake, as suggested by several authors (Le Bot *et al*., 1990; Kirk and Le Ven Du, 1997; Neumann and Römheld, 1999; Neumann *et al*., 1999; Hinsinger *et al*., 2003). Many of the previous works that have addressed the response of plant roots to P deficiency have however been conducted with rather extreme situations where P-deficient plants were not supplied with any P at all for days or weeks. In the present work, we compare the response of plants that received two levels of P, one of which was conducive to P deficiency. The present study aimed at comparing the release of protons by two bean genotypes (BAT477 and DOR364) relying on various sources of N, and its response to P deficiency. This was assessed both at the whole plant level via a pH-stat experiment conducted in hydroponic culture and at the single root level via the dye indicator-video densitometry technique.

These two methods are designed so that pCO_2 cannot build up in the vicinity of the roots in spite of rhizosphere (root and microbial) respiration, and therefore the latter process cannot contribute any significant pH decrease under such conditions (Jaillard *et al*., 2003). Thereby, it can be assumed that all observed pH changes are accounting for proton/ hydroxyl release to counterbalance cation-anion uptake and/ or organic anion exudation (Hinsinger *et al*., 2003).

Nitrogen (N_2)-fixing plants have an inherently higher phosphorus (P) demand than NO_3 –fed plants, which attributed to the high energetic cost of symbiotic N2 fixation (SNF) (Ribet & Drevon, 1995). This is particularly true for common bean which is generally considered to be more sensitive than other legumes crops to most biotic and abiotic contrainst, including P deficiency (Vadez *et al.,* 1996). Nodulation requires large allocation of plant P resources, nodule P concentration being up to 3-fold higher than that of the other organs (Jakobsen, 1985; Vadez *et al.,* 1999). A positive correlation between nodule number and P availability has been often documented, P deficiency leading to nodulation delay and impairment (Araujo ans Teixeira, 2000; Kouas *et al.,* 2005). Nodule growth is more sensitive to P deficiency than plant growth (Drevon & Hartwig, 1997). Common bean lines, (Kouas *et al.,* 2005) showed that nodule number and biomass were significantly diminished by low-P treatments. The present work reports the differences for P acquisition and its onward utilization for producing biomass among six common bean genotypes .To measure phosphorus use efficiency by genotypes for N2 fixation under controlled conditions of rhizobox.

Materials and Methods

Growth conditions

Six common bean genotyes of (*Phaseolus vulgaris* L.) were selected among the progenies of the crossing of two parental RILs, namely BAT477 and DOR364. The seeds were sterilized with 3% calcium hypochlorite for 5 min., and rinsed by 5 washings with sterile distilled water. They were then transferred for germination on soft agar, consisting of 100 ml Bergersen solution containing 5 g mannitol and 7 g agar in 1: l of distilled water with sterilization at 120°C for 20 min., (Vincent, 1970). The inoculation was performed by soaking 4 d-old seedlings for 30 min. in a suspension of an inoculum of *Rhizobium tropici* CIAT899 containing 109 bacteria ml^{-1}. This rhizobia, kindly provided by the CIAT, is a reference for the studies on common bean because of its high capacity to fix nitrogen and its ubiquity with the diversity among *P. vulgaris* (Vadez *et al.,* 1996). The inoculum was prepared from rhizobia culture preserved in tubes at 4°C, on the following 120°C sterilized agar YEM (Yeast Extract Mannitol) medium: 900 ml distilled water; 100 ml of Bergensen concentrated solution containing 1 g KCl; 0.1 g $FeCl_3$; 0.4 g $CaCl_2$, 4.5 g Na_2HPO_4-12H2O and 1 g $MgSO_4$ -$7H_2O$, firstly in 100 ml of distilled water then adjusted to 1 l, and subsequently added with 1 g Yeast extract, 10g mannitol and 15g agar (Vincent, 1970). Rhizobial colonies were transferred from preservation tubes into 100 ml of liquid YEM with stiring at 28 °C during 24 h. For each P treatment, 20 inoculated plants were transferred into 45 l vat, 0.2 m large, 0.4 m long and 0.4 m high for hydroaeroponic pre-culture during 28 d. Based on work of Vadez *et al.,* (1996). P was supplied weekly in the form of KH_2PO_4 (75 or 250 ìmol $plant^{-1}$ $week^{-1}$ for deficient or sufficient P supply) to the following nutrient solution that was changed every week: $CaCl_2$ (1650 mM); $MgSO_4$-$7H_2O$ (1000 mM); K_2SO_4 (700 mM); Fe EDDHA (8.5 mM Fe as sequestrene); H_3BO_3 (4 mM); $MnSO_4$- H2O (6mM); $ZnSO_4$-$7H_2O$ (1 mM); $CuSO_4$-$7H_2O$ (1 mM); Na_2MoO_4-$7H_2O$ (0.1 mM). The oxygenation of the culture solution was ensured by a permanent flow of 400 ml^{-1} min^{-1} of compressed air. The pH was adjusted daily to a value of 6.8 with KOH (0.1 M). A supply of urea was provided with 2 mmol $plant^{-1}$ in the initial solution and 1 mmol $plant^{-1}$ at the first change of solution after two weeks, in order

to optimize nodulation (Hernandez & Drevon, 1991). The plants were then grown in N-free nutrient solution The whole experiment was carried out in a glasshouse under temperature conditions of 28/20 °C during 16/8 h day/night cycle with an additional illumination of 400 mmol photons m^{-2} s^{-1} and 70% relative humidity during the day.

Experimental set up

The experiment was conducted in INRA-Montpellier glasshouse in the south of France during the year 2008-2009. Under temperature conditions of 28/20 °C during 16/8 h day/night cycle with an additional illumination of 400 µmol photons m^{-2} s^{-1} and 70% relative humidity during the day. Seeds of six common bean lines (*Phaseolus vulgaris* L.) RILs 34, 75, 83, 104, 115, 147 were used in this experiment. The experiment was set up with two P levels (250 and 75Pµmol pl^{-1} $week^{-1}$) and six RILs of (*Phaseolus vulgaris* L. Treatments were replicated nine times and completely randomized.

Soil analyses

Table 1 shows Physical and chemical properties of cazevieille soil of rhizobox, the soil was characterized by high cation exchange capacity (CEC), neutral pH and low content of available P, in spite of its rather large total P content (Alkama *et al.*, 2009). It was sampled at a depth of 5–20 cm in Cazevieille (South of France), and was sieved (<2 mm) after removing stones and plant residues. It is classified as a fersiallitic soil, i.e. chromic cambisols according to FAO–UNESCO (1989). A polyamide mesh of 30 mm (Nytrel 0.2SPN, Fyltis-U.G.B., and Lyon, France) separated the soil from roots without limiting the exchange of water and chemical with the nodulated roots (Hinsinger & Gilkes, 1997). Each 24 g of soil used in each rhizoboxes was incubated for 4 d at 20 °C. The rhizoboxes were fixed vertically into buckets, with a filter paper as wick bathing in the previously described nutrient solution. The initial pH of the soil was measured in an aqueous suspension with a soil/water ratio of 1/5 (v/v), after having calculated the water content of each sample. At harvest, a fraction of soil of each replicate was weighed then dried at 105 °C for 24 h to estimate the water content of each soil sample. The H^+ efflux, expressed in mmol $plant^{-1}$ d^{-1}, was calculated as: QH^+ ($\beta s * \Delta pH * Ma$)/ t^{-1} where, βs is buffer capacity of the soil in mmol OH^- g^{-1} soil unit pH, ΔpH is difference between the final pH at harvest and the initial pH before the culture, Ma is mass of soil used in g, t is the duration of culture in d (Helyar & Porter, 1989). The soil buffering capacity was assessed by decreasing or increasing soil pH by 1 unit after addition of a solution of H_2SO_4 or KOH. According to the proton balance, the soil pH depends upon the amount of H^+ added or depleted from soil solution and the intensity of the soil buffer that depends on the contents of clay and organic matter (Conyers *et al.*, 1995).

Plant analyses

Phosphorus concentration in shoot and root digest was estimated using vanadate-molybdate colorimetric method (Chapmann and Pratt, 1961). Phosphorus contents (mg P $plant^{-1}$) were calculated in root and shoot by multiplying P concentration in the respective tissue with its dry matter and on whole plant basis by adding up shoot and root P contents. Phosphorus utilization efficiency (g SDW mg^{-1} P) was calculated by the following formula (Siddiqui & Glass, 1981) Phosphorus Utilization Efficiency = 1 / Shoot P concentration X Dry matter

Harvest and statistical data processing

The plants were harvested at the flowering stage with the first pod measuring 2 cm long. The shoot was separated from the root at the cotyledonary node, then weighed after 48 h at 70 °C. Nodules were separated from the roots,

Table 1: Physical and chemical properties of cazevieille soil of rhizobox.

Characteristic	Value	Characteristic	Value
Clay(%)	48,5	CEC (cmol kg^{-1})	21.6
Fine silt(%)	21.8	Ca (g/ kg)	3.7
Croase silt (%)	17.8	Na kg	0,13
Fine sand(%)	11.6	Mg (cmol kg^{-1})	1.00
Coarse sand (%)	3	K g/ kg	0.179
pH H_2	6.9	Somme Base (cmol kg^{-1})	20
Buffering capacity of soil	54	% Saturation	93
$CaCO_3$(g kg^{-1})	1	P total (g/100g)	208
C organic (g kg^{-1})	24.1	P Olsen(g/kg)	0.007

counted and weighed separately. Soil samples were dried at 105 °C for 24 h. Their pH was measured in an aqueous suspension shaken at 1200 rotations min-1 for 30 min with a soil/solution ration of 1/5. The available P of the soil was measured by the Olsen method according to standard NF ISO 11263 after extraction by soil agitation with a solution of 0.5 N sodium bicarbonate with pH 8.5. In order to determine the contribution of P supply to plant growth, the response curves were established from biomass values with the software Excel Microsoft Office. For regressions shown in various figures, covariance analysis was performed for the calculation of the F value, with decision of significance for $p<0.05$. Differences between means of all parameters for sufficient versus deficient P treatments were determined by two-way analysis of variance (ANOVA) and significance was tested with the Fisher's LSD test, with $p<0.05$, $P<0.01$ as significant and highly significant, respectively.

Results and Discussion

Plant growth and nodulation

Shoot biomass is the best growth indicator parameter for screening purpose because it directly indicates P nutrition effect on plant .Common bean genotypes (Table 2) showed significant differences in shoot biomass at both P-levels. With sufficient P-level, shoot biomass ranged from 4.14 to 4.60 g plant^{-1} and at deficient P-level, it ranged from 3.64 to 4.32 g plant^{-1}. Maximum and minimum shoot biomass were observed in genotype 75 (4.60 g plant^{-1}) and genotype 147(4.14 g plant^{-1}) at sufficient P-level; whereas, with deficient P supply, genotype,75 exhibited maximum (4.32 g plant^{-1}) and genotype 115 showed minimum shoot biomass (3.64 g plant^{-1}), respectively. Wide differences in shoot biomass at deficient P-level suggest differential biomass production behavior of common bean genotypes, which can be exploited for further selection, and recommendation of common bean genotypes for areas deficient in soil-P. The growth of plant is diverted from shoot to root under P-deficiency stress. This adaptive mechanism is due to change in internal physiology of the plant (Horst *et al.*, 1993). Data on RDW (Table 2) exhibited significant differences at both P-levels. At sufficient P-level, it ranged from 1.04 to 1.24 g plant^{-1} and at deficient P-level; it ranged from 1.05 to 1.26 g plant^{-1}. Maximum root biomass was observed in genotype 83 (1.24 g plant^{-1}) followed by genotype 34 (1.21 g plant^{-1}) and genotype 75 (1.17 g plant^{-1}); whereas, minimum root biomass was produced by genotype 115 (1.04 g plant^{-1}). P supply and genotypes affected significantly on shoot biomass especially for genotypes 83 and 75 (fig. 1a) shows that. On the other hand (Fig. 1b) shows no significant effect of p supply and genotypes on root biomass at deficient or sufficient P. Under P sufficiency, the nodule biomass was significantly higher for genotypes 34,83 and104 than for the other RILs (Fig. 1c) shows that. At sufficient P-level, nodule biomass ranged from 0. 41 to 0.56 g plant^{-1} and at deficient P-level, it ranged from 0.23 to.38 g plant^{-1}. Maximum and minimum nodule biomass were observed in genotype 34 (0.56 g plant^{-1}) and genotype 115 (0.41 g plant^{-1}) at sufficient P-level; whereas, with deficient P supply, genotype 34 exhibited maximum (0.38 g plant^{-1}) and genotype 104 showed minimum shoot biomass (0.23 g plant^{-1}), respectively in (table 2). The P supply and genotypes affected significantly on nodule number, with the highest nodule numbers in genotypes 34 and 83 and the lowest in genotype 147 at sufficient P (Table 2) and (Fig. 1d) show that. At deficient P the highest nodule numbers was

Table 2: Shoot biomass, root biomass, nodule biomass and nodule numbers of six common bean genotypes (*Phaseolus vulgaris* L.) grown under sufficient and deficient phosphorus (250 vs. 75 P) in rhizobox.

Genotypes	Shoot biomass g plant^{-1}		Root biomass g plant^{-1}		Nodule biomass g plant^{-1}		Nodule numbers nod plant^{-1}	
	250P	75P	250P	75P	250P	75P	250P	75P
34	4,30ab± 1.01	3,95ab±0.81	1,21a±0.35	1,16a±0.14	0,56a±0.26	0,38abc±0.19	399a±117	338ac±167
75	4,60a±1.18	4,32ab±0.80	1,17a±0.20	1,13a±0.27	0,44ac±0.24	0,32bc±0.15	299a-c±149	259a-c±128
83	4,38ab±0.95	3,66b±0.58	1,24a±0.28	1,17a±0.26	0,56a±0.31	0,34bc±0.16	356ac±143	251bc±238
104	4,15ab±0.67	3,69b±0.69	1,07a±0.23	1,05a±0.23	0,44ac±0.25	0,23b±0.13	297a-c±059	188b±125
115	4,04ab±0.91	3,64b±1.23	1,04a±0.18	1,10a±0.35	0,41a-c±0.21	0,29bc±0.18	317a-c±190	224bc±231
147	4,14ab±1.04	4,08ab±1.22	1,08a±0.26	1,26a±0.47	0,46ac±0.14	0,31bc±0.23	271a-c±189	277a-c±150
P rate	**		N.S		**		**	
Genotypes	*		N.S		*		*	

Whereas * = significant at $p<0.05$, ** = significant at $p<0.01$ and ns= non significant

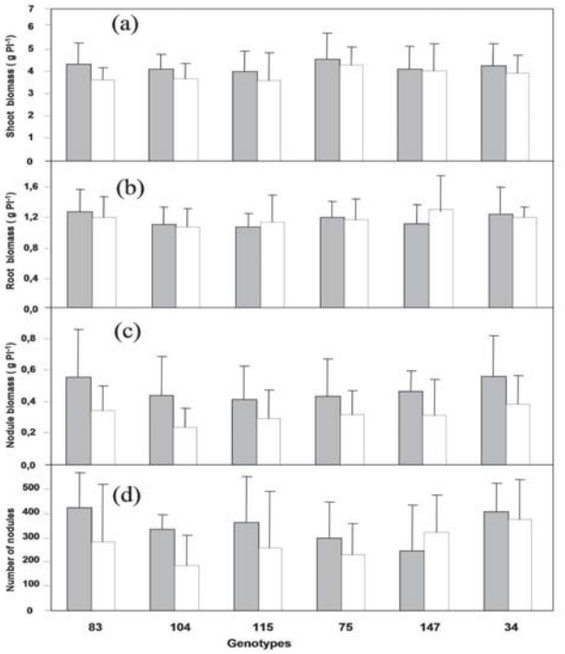

Fig. 1: Effect of P nutrition on a) shoot b) root biomass c) nodule biomass and d) nodule number in six bean genotypes in rhizobox. Data are means and SD of 9 harvested plants at 45 day after sowing. P sufficiency versus P deficiency

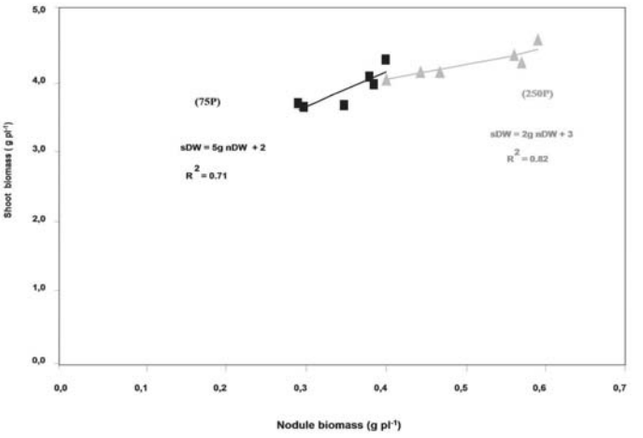

Fig. 2: Effect of P nutrition on use efficiency of symbiotic nitrogen fixation (EUSR) (the regression parameter of shoot as a function of nodule) of the genotypes in rhizobox. Significant regression was found with lower EUSR under P deficiency than under P sufficiency. Data are means SD of 9 replicates harvested at 45 days after sowing.

observed in genotype 34 (338 nod. Plant^{-1}) and the lowest value was observed in genotype 104 (188 nod. Plant^{-1}).

Correlation between shoot and nodule biomass

In order to assess the efficiency in use of the rhizobial symbiosis (EURS), the values of the shoot biomass were plotted as a function of those of nodule biomass in Fig. 2 with the slope of the regressions being an estimate of the EURS. Significant regressions were found for genotypes at sufficient P more than at deficient P deficiency whereas r = 0.91 at sufficient P and r = 0.84 at deficient P.

Shoot P concentration and efficiency

P supply and genotypes affected significantly on shoot P content and there were significant differences in (Table 3.) and shown in (Fig.3a) among genotypes for their P-concentration at sufficient P-level in shoot content more than deficient P. Whereas significant differences were observed among genotypes at sufficient P-supply. Maximum and minimum P-concentration was observed in shoot, were observed in genotype 147 (1.84 mg P g^{-1}) followed by genotype 75 (1.83 mg P g^{-1}) and genotype 104 (1.76 mg P g^{-1}) and minimum was observed in genotype 83 (1.24 mg P g^{-1}), respectively. Whenever at deficient P level no significant differences were observed between genotypes. P utilization efficiency refers to the biomass production per unit of tissues P concentration. It demonstrates the efficient and non-efficient behavior of species towards P utilization (Siddiqui & Glass, 1981). Data regarding PUE (Fig 3b) shows that the most efficient genotype was 147 followed by genotypes 104 and 115 respectively, at deficient P-level. However, with sufficient P, genotype 34 was the best utilizer of P followed by genotype 83 and genotype 115. (Fig. 3c) shows that genotype 75 the highest uptake P at sufficient and deficient P followed by genotypes 147 and 104 respectively.

Nodule P concentration and efficiency

Table 3. illustrates that there is a significant affected of P supply on nodule P content at sufficient and deficient P

Table 3: Shoot P concentration,, nodule P concentration Soil Olsen P and Soil pH of six common bean genotypes (*Phasolus vulgaris* L.) grown under sufficient and deficient phosphorus (250 vs. 75 P) in rhizobox.

Genotypes	Shoot P concentration (mg Pg^{-1})		Nodule P concentration (mg Pg^{-1})		Soil Olsen P (mg kg^{-1})		Soil pH	
	250P	75P	250P	75P	250P	75P	250P	75P
34	1.26bc±0.02	1.19c±0.02	2.35a-d±0.06	1.80bd±0.09	23.00ab±7.12	12.00c±3.06	6,49a±0.26	6,58a±0.14
75	1.83a±0.02	1.23bc±0.03	2.40a-c±0.05	2.10a-d±0.05	23.00ab±8.25	10.00c±2.89	6,52a±0.16	6,56a±0.17
83	1.24bc±0.03	1.17c±0.02	2.50ac±0.10	1.70d±0.08	20.00b±8.12	10.00c±3.84	6,63a±0.14	6,49a±0.19
104	1.76a±0.10	1.22bc±0.01	2.60ac±0.07	2.00b-d±0.08	20.00ab±5.90	10.00c±3.67	6,60a±0.10	6,60a±0.21
115	1.53ab±0.07	1.04c±0.01	2.70a±0.08	1.70d±0.04	20.00b±4.44	9.00c±4.42	6,69a±0.15	6,59a±0.17
147	1.84a±0.06	1.08c±0.01	2.30a-d±0.11	2.00b-d±0.09	26.00a±7.57	12.00c±4.64	6,55a±0.11	6,64a±0.07
P rate	**		**		**		N.S	
Genotypes	**		N.S		**		*	

Whereas * = significant at p<0.05, ** = significant at p<0.01 and ns= non significant

with the higher values under sufficient P than deficient P. Whereas at sufficient P the highest nodule P content was observed in genotype 115 (2.70 mg P g^{-1}) followed by genotypes 104 (2.60 mg P g^{-1}) and 83 (2.50 mg P g^{-1}) respectively. At deficient P the highest nodule P content was observed in genotype 75 (2.10 mg P g^{-1}) followed by genotypes 104 (2.00 mg P g^{-1}) and 147 (2.00 mg P g^{-1}) respectively. Meanwhile no significant effect of genotypes was observed at sufficient or deficient P, (Fig. 4a) shows that whereas at sufficient P level all genotypes have a higher values than at deficient P level. Fig. 4b shows. No differences between the genotypes for P utilization at

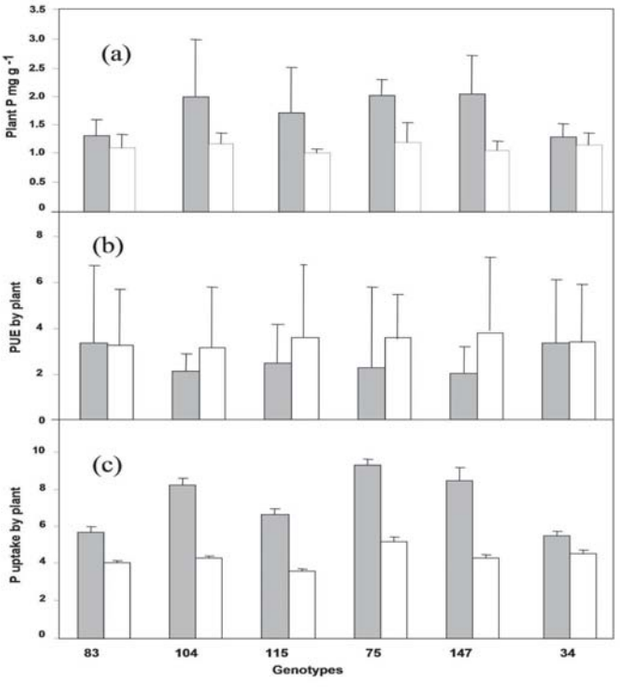

Fig. 3. Effect of P nutrition on a) shoot and b) phosphorus use efficiency and c) p uptake by plant of six common bean genotype at flowering stage in rhizobox. Data are means and SD of 9 replicates harvested at 45 days after sowing. P sufficiency versus P deficiency

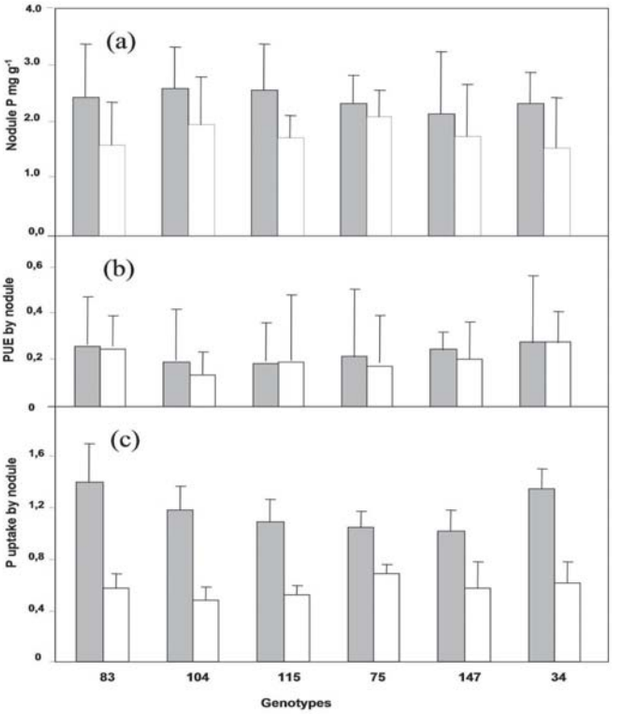

Fig. 4. Effect of P nutrition on a) nodule and b) phosphorus use efficiency and c) p uptake by nodule of six common bean genotype at flowering stage in rhizobox. Data are means and SD of 9 replicates harvested at 45 days after sowing. P sufficiency versus P deficiency

sufficient and deficient P and the efficiency in utilization P by common bean genotypes under both levels were low. By contrast the P uptake by plant was high significantly at sufficient than deficient P. Whereas the highest genotype uptake of P was observed by genotype 83 followed by genotypes 34 and 104 respectively at sufficient P, and under deficient P the highest genotype uptake P was 75 followed by genotypes 34 and 147 respectively.

H^+ efflux by nodulated-roots

RILs affected significantly the pH of the rhizosphere whereas P supply had no effect. Under P sufficiency, 34, 75 and 147 induced the lowest pH, whereas under P deficiency, 75, 83 and 34 induced the lowest pH in contrast with 147 with a significant difference of about 10% between RILs (Fig 5a). The data in figure 5a made it possible to calculate H+ efflux plant^{-1} d^{-1} and H^+ efflux rDW^{-1} d^{-1} from soil buffering capacity as described in materials and methods. Under P sufficiency there was more H+ efflux plant^{-1} d^{-1} for 34,75 and 147 than for 104, 83 and 115 (Fig. 5b), and the H^+ efflux rDW^{-1} d^{-1} for 34, 147 and 75 was significantly higher than for 115, 83, and 104 by more than 30%. Under P sufficiency 34 was significantly higher than, 147 and 75 by more than 20% (Fig5b).Considering the two P treatments, nodule biomass was positively correlated with Olsen P (Fig. 6a). (r^2 = 0.88) We noted that genotypes had increased their nodule biomass under P sufficiency more than under deficiency, where this parameter was slightly low under P deficient. Also, significant correlation (r^2 = 0.81) was found between Olsen P and nodule number under P sufficiency. By contrast this correlation was weak under P deficiency (r^2 = 0.35). Moreover, correlation between shoot biomass and Olsen P (Fig. 6c) had approximately the same trend of that observed between nodule number and Olsen P. As it was well documented in hydroponic system which allows studying in nutrient solution several parameters linked to nodulated roots tolerance against P deficiency, rhizobox system with rhizoheric approach seems to be more appropriate in examination of both rhizosphere and nodulated roots. In this study, rhizobox system enabled us to better exanimate behaviour of several RILs of common beans under P deficiency. To our knowledge, tolerance of these RILs against P deficiency resulted in an increase both in protons release and available P of the rhizospheric soil. Furthermore, changes of the rhizospheric soil pH constitutes one of the main results of this work which significantly varied among genotypes (P<0.05) (Table 3). This acidification as well proton efflux was associated with a significant (P<0.01) increase of Olsen P that showed differential behaviours at the level of growth and nodulation. Interestingly, increasing

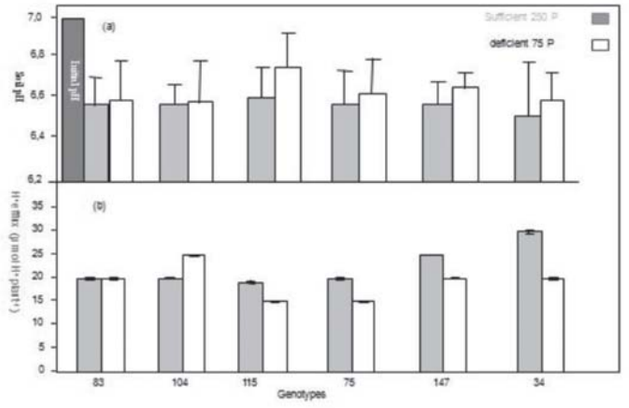

Fig. 5. Effect of P nutrition on a) pH, b) mean of H^+ efflux per plant, c) of six common bean genotypes in rhizobox. Data are means SD of 9 replicates harvested at 45 days after sowing.
P sufficiency versus P deficiency

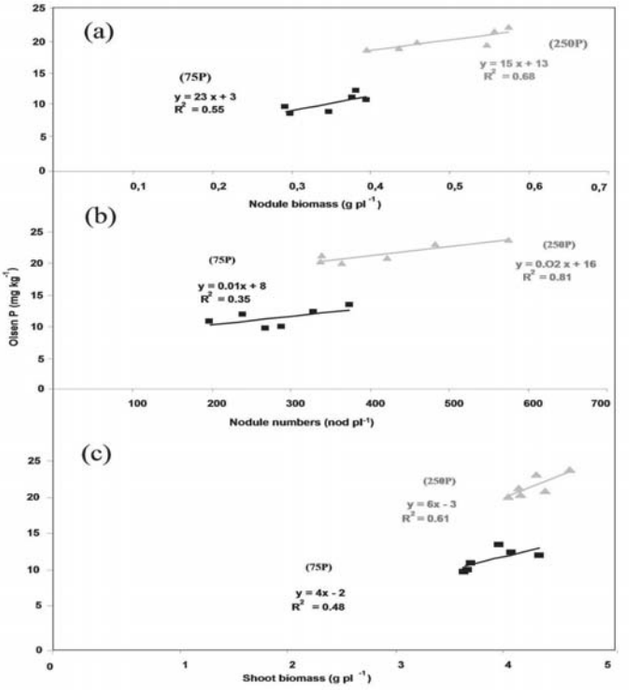

Fig. 6. Correlation between available P (Olsen P mg kg-1) and a) nodule biomass (g plant^{-1}), b) nodule numbers (nod. Plant^{-1}) and c) shoot biomass (g plant^{-1}) of six common bean genotype at flowering stage in rhizobox. Data are means and SD of 9 replicates harvested at 45 days after sowing. P sufficiency versus P deficiency.

P in the rhizosphere resulted in an increase in nodulation and plant growth (Fig. 6b and c). However this relation is not always true if we consider analysis of genotypes individually.

Variation we found at the rhizosphere might affect plant and nodule physiology as swell as P content and PUE which are described to be involved in P deficiency tolerance for SNF. In this study, more variations were found in shoot P than in nodule P content (Table 3). Although this parameter was decreased under P deficiency for all the RILs, differences were not significant. The high P allocation in nodules that is almost two times high than P shoot may be involved in P tolerance of this symbiosis since P is highly requested for N_2 fixation. Moreover, (talk about roles of P for nodule respiration and nodule formation as well as bacteroides demand). Increase of P in nodule has been described to be linked with increase of the nodule conductance to the O_2 diffusion (Bargaz *et al*., 2011). Furthermore, increases of P both in shoot and nodule were associated with an increase (60%) in EURS (Fig. 2) under P deficiency than under P sufficiency. We suggest that P distribution is highly regulated inside the whole studied symbiosis and is in perpetual exchange with the rhizosphere component as well as available P. Hence, this is in agreement with the positive correlation we found between Olsen P and both nodule and shoot biomasses (Fig. 6). So The aim of this research was to investigate the role of common bean RILs in use of phosphorus to enhance their nodulated roots to reduce pH and increase H+ efflux thus increase available P in rhizobox. Also address whether a P-sufficient genotype may have more proton efflux than a P deficient. Some differences in P-requirements between the RILs was observed in (Fig. 1,3 and 4). The greater tolerance RIL were 34, 75 and 147 compared to others is related to a greater phosphorus use efficiency (PUE) that is illustrated by greater growth of nodules, shoot and roots with the same amount of available P (Table 2,3). The greater P requirement for nodules than shoot agrees with the previous observation that the P content is larger in nodules than in shoots for *A. mangium* (Ribet and Drevon, 1996) and *G. max* (Ribet and Drevon, 1995). The high requirement of P for nodules might be related to high energy requirement of the SNF process (Israel, 1987), i.e. the equivalent of 30 ATP per N2 reduced (Salsac *et al.*, 1984).

Conclusion

The results we had obtained in this work meet the main objective of the work which is increasing the bio-availability of P in soil by common bean genotypes (*Phaseolus vulgaris* L.) contrasting for phosphorus by release H^+ efflux by their nodulated roots. The greater H^+ efflux for the P-sufficient than for the P-deficient agrees with previous conclusion of Tang *et al.*, (2001a) with (*Phaseolus vulgaris)*. The greater H^+ efflux under P sufficiency than deficiency is consistent with the finding in genotypes of *G. max* (Tang *et al.*, 2007). The acidification of the common bean rhizosphere could be related to an excess of cation absorption (Tang, 2001a; Hinsinger *et al.*, 2003). The rhizospheric acidification could contribute to the adaptation of legume genotypes and species to P deficiency through the effects on P bioavailability. In the case of soil in rhizobox , the P that is fixed in the calcic-phosphatic complexes can be solubilized according to the following equation for hydoxyapatite which shows the solubilizing effect of H^+ (Hinsinger and Gilkes, 1997): $Ca_{10}(PO_4)_6(OH)_2 + 12H+ 10Ca^{2+} + 6(H_2PO_4)^- + 2(OH)^-$ .Further work is needed to adapt the rhizobox device to the large tap-root, in order to explore the relationships between H^+ efflux and P solubilization in common bean rhizosphere with a larger collection of contrasting genotypes.

Acknowledgements

This work was supported by INRA-SUPAGRO Montpellier during the experiment and the laboratory analyses. The author is grateful to Eng. Vailhe H for her help and efforts throughout this work and National Research Centre for facilitating the tasks during these experiments.

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