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Original article

Genotypic variability in phosphorus use efficiency for symbiotic N_2 fixation in common bean (*Phaseolus vulgaris*)

Vincent VADEZ[a], Jean-Jacques DREVON[b]*

[a] CIAT, Centro Internacional de Agricultura Tropical, Apdo Aereo 6713, Programa de Nutricion Frijol, Cali, Colombia
[b] Laboratoire de Recherche sur les Symbiotes des Racines, INRA (Institut National de la Recherche Agronomique), place Viala, 34060 Montpellier Cedex, France

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Abstract – Three bean (*Phaseolus vulgaris* L.) genotypes (APN18, BAT271, G12168) were inoculated with a mixture of 3 rhizobia (*R. etli* CIAT632 and CIAT7115 and *R. tropici* CIAT899) and grown either with 10 mmol NO_3^-·week^{-1} (NO_3-fed) or with N_2 fixation (N_2-dependent) in an aerated nutrient solution under various P supplies. Phosphorous supplies for maximum plant growth varied between genotypes, and were always lower with N_2 than with NO_3. By contrast with NO_3-fed plants, N_2-dependent plants accumulated highly contrasting amounts of N at low versus high P supplies (72 versus 360 µmol P·plant^{-1}·week^{-1}). Under low P, N_2-dependent APN18 and BAT271 fixed respectively 2 and 3 times more N_2 than G12168, although nodule dry weight was similar in these genotypes. The ratio N accumulation:plant P concentration (PUEN) showed large genotypic contrast under low P supply. It is concluded that these genotypic differences depend on the efficiency in use of P for N_2 fixation, which is linked to the partitioning of P in plants, and that PUEN may be used to screen for genotypic variation of N_2-dependent growth of legumes under low P supply.

N_2 fixation / *Phaseolus vulgaris* / phosphorous / *Rhizobiaceae* / symbiosis

Résumé – Variabilité génotypique de l'efficacité d'utilisation du phosphore pour la fixation symbiotique d'azote chez le haricot (*Phaseolus vulgaris*). Trois génotypes de haricot (*Phaseolus vulgaris* L.) (APN18, BAT271, G12168) ont été inoculés avec un mélange de 3 rhizobia (*R. etli* CIAT632 and CIAT7115 and *R. tropici* CIAT899), et cultivés en hydroaéroponie soit avec 10 mmol NO_3^-·semaine^{-1} ou avec la fixation de N_2, sous divers apports hebdomadaires de P. La croissance était maximale pour des niveaux de P différents selon les génotypes, en étant toujours inférieure pour les plantes dépendant de la fixation de N_2. Celles-ci, contrairement aux plantes dépendant de NO_3, accumulaient des quantités différentes d'azote à faible ou fort P (72 versus 360 µmol P·plante^{-1}·semaine^{-1}), ce qui indique une relation spécifique entre la fixation de N_2 et la nutrition P. A faible P, APN18 et BAT471 fixaient 2 à 3 fois plus de N_2 que G12168, avec des masses nodulaires similaires, mais un ratio N accumulation:P concentration (PUEN) variant significativement selon les génotypes. Il est conclu que ces différences génétiques pour la fixation N_2 sous bas P dépendent de l'efficacité d'utilisation du P, qui est liée à la répartition du P entre les organes, et que le ratio PUEN peut être utilisé pour sélectionner des lignées plus tolérantes à la carence P.

fixation d'azote / *Phaseolus vulgaris* / phosphore / *Rhizobiaceae* / symbiose

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* Correspondence and reprints
drevonjj@ensam.inra.fr

1. INTRODUCTION

Phosphorous is often a primary factor limiting plant growth, especially in tropical soils. Furthermore, P deficiency is more likely to affect N_2-dependent legumes than other species because symbiotic nitrogen fixation is an energetically expensive process which requires more inorganic P than mineral nitrogen assimilation [16]. In bean, a crop widely grown in P-deficient tropical soils, production may be highly affected by low availability of soil P, especially when the latter is accompanied by low availability of soil N, thereby forcing plants to rely on N_2 fixation.

There are two potential physiological approaches to improve plant growth and yield under low soil P availability [5], namely efficient uptake of external P, and efficient utilization of internal P. The first approach involves plant-soil interactions such as modification of soil exploration by roots, improved interactions with soil microorganisms such as mycorrhizal fungi, and rhizosphere modification to increase P availability [2, 6, 13, 14, 23]. The second approach involves efficient partitioning and subsequent utilization of P within the plant, resulting in more biomass produced and N_2 fixed per unit of P taken up. This latter strategy has not been extensively studied although various plant species differ for internal P requirements [8].

Investigations have been carried out on the P requirements for N_2-fixation in legume crops like cowpea (*Vigna unguiculata*) [4], pea *(Pisum sativum)* [18], soybean (*Glycine max* L. Merr.) [16] and *Acacia mangium* [27, 31], showing that P requirements are generally higher for N_2 fixation than for shoot growth and mineral N assimilation, since nodules are an additional strong sink for P. Furthermore P requirements for N_2 fixation varies between genotypes in pigeon pea (*Cajanus cajan*) [1] and mungbean (*Vigna radiata*) [11], or *Casuarina–Frankia* symbioses [29]. Differences in N_2 fixation related to the efficiency in utilization of P were found among soybean genotypes [11], and *Acacia mangium* populations [32]. According to Cassman [4], efficient P utilization in N_2-fixing symbioses may be closely related to an adequate P partitioning between shoot and nodulated root, and between root and nodules.

Two ratios are commonly used for the calculation of P use efficiency [9]: (i) the utilization quotient (UQ), defined as the ratio biomass:biomass P content, which is equal to the reciprocal of P concentration in biomass, and the P use efficiency ratio (PUE), defined as the ratio biomass:biomass P concentration. High UQ ratios are often obtained at very low P because biomass P concentration tends to decrease and, therefore, UQ expresses a dilution effect of P rather than an actual efficiency in P utilization when P is very limiting [17]. By contrast, the PUE ratio takes into account the nutrient concentration together with plant growth and states that a nutrient is needed at a threshold concentration to reach maximum growth [17]. Therefore, this ratio is considered as a more reliable estimate of P use efficiency [30]. Attempts have been made to increase bean productivity by selecting lines able to fix adequate N_2 under conditions of low P availability [24, 25]. However, no specific attention has been given to looking at bean lines with a high PUE.

The objectives of the present work were to test whether critical levels of P nutrition were higher under N_2-fixing conditions than under mineral N nutrition for a range of bean genotypes and to determine the extent of intraspecific variability in PUE for N_2 fixation in common bean. Additionally, the partitioning of P in these plants was investigated as an attempt to identify the potential mechanisms for adaptation of N_2 fixation to low P availability.

2. MATERIALS AND METHODS

2.1. Plant growth in hydroponic culture

Trials were performed in a temperature-controlled glasshouse with night/day temperatures of 25/33 °C and a 13 h photoperiod at an elevation of 1000 m. Bean seeds were pre-germinated for 6 days in trays containing vermiculite moistened with deionized water, and inoculated with 100 ml of a mixture of *Rhizobium etli* (CIAT 7115, CIAT 632) and *R. tropici* (CIAT 899) containing approximatively 10^8 cells·mL^{-1}. At transplanting, seedlings with uniform root-size were gently passed through the hole of a rubber stopper with cotton wool at the hypocotile level to prevent the stem from slipping through. The rubber stopper was then fitted to the bottle neck, allowing the root system to dip into the nutrient solution.

Plants were grown in 900 mL glass bottles, wrapped with aluminium foil to maintain darkness in the rooting environment, containing the following nutrient solution: $CaCl_2$ (3.3 mM), $MgSO_4$ (2.05 mM), K_2SO_4 (1.25 mM), H_3BO_3 (4 µM), $MnSO_4$ (6.6 µM), $ZnSO_4$ (1.55 µM), $CuSO_4$ (1.55 µM), $CoCl_2$ (0.12 µM), $NaMoO_4$ (0.12 µM), FeEDTA (40 µM) [7]. This solution was replaced for the first time 10 days after sowing and thereafter once weekly. The nutrient solution was constantly aerated at a flow of 400 mL·min^{-1} and its pH was maintained near 7 through addition of $CaCO_3$ (1 g·L^{-1}).

2.2. Experimental design and treatments

Phosphorous requirements were investigated under two types of N nutrition, N_2 fixation or nitrate supply. Based on results obtained in a preliminary experiment, APN18, BAT271 and G12168 were chosen as representative of a larger range of genotypes [33], and were grown in the described system at 6 levels of P supply in a randomized complete block design with 3 replicates.

The N_2-dependent plants were supplied with 2 mM urea as starter nitrogen at transplanting and 1 mM at the first nutrient solution replacement, and were grown without N in the nutrient solution after 17 DAS (days after sowing). The NO_3-fed plants were treated similarly during the first 17 DAS and then received 10 mM KNO_3 at each weekly nutrient solution replacement. The six P treatments were applied as KH_2PO_4 equivalent to 45, 72, 135, 220, 360, 540 µmol P·plant^{-1}·week^{-1}.

2.3. Tissue analysis and statistics

The plants were harvested 50 DAS, at the early pod-filling stage, and separated into shoot, root and nodule (for N_2-fixing plants) fractions which were then dried at 60 °C to constant weight. Each fraction was then ground, wet digested at 350 °C in a mixture of concentrated nitric and perchloric acid for P determination according to [22] and in concentrated sulfuric acid for N determination by Kjeldahl analysis. Nitrogen accumulation was expressed after deduction of the starter nitrogen supplied as urea (3 mmol per plant corresponding to 84 mg N) after confirming on a separate set of plants that all starter urea had been taken up by plants (data not shown).

The GLM procedure of SAS (Statistical Analysis System) was used to perform the analyses of variance of the block design, and model regression as linear or parabolic functions. Significance is given at the 95% level.

3. RESULTS

3.1. Shoot, root and nodule biomass

Genotypic differences of shoot biomass in response to varying P supply were significant only with N_2-dependent plants (Fig. 1a, b): in APN18, shoot response to P was low, maximum biomass being reached at 135 P (135 µmol P·plant^{-1}·week^{-1}), for a maximum of 10.9 g DW shoot·plant^{-1}; in BAT271, shoot biomass increased up to 220 P with a maximum of 18.6 g DW shoot·plant^{-1} (i.e. the highest among the genotypes), while in G12168 the response of biomass to P supply was moderate with a response to P supply up to 360 P (Fig. 1a).

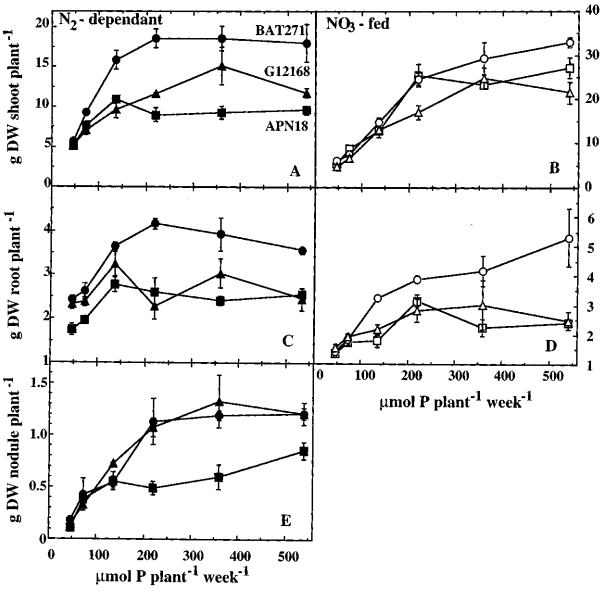

Figure 1. Effect of P supplies on shoot (a, b), root (c, d) and nodule (e) dry weight in bean genotypes (BAT271, G12168, APN18) grown with NO_3 (opened symbols) or with N_2 fixation (closed symbols). Data are the means ± SE of 3 replicated plants, harvested at 50 DAS.

With NO_3, critical P supplies for shoot biomass were at least equal to (G12168) or higher than (APN18 and BAT271) those observed with N_2 fixation, and maximum biomass was always higher than with N_2 fixation (Fig. 1b), varying between 21.5 and 32.9 g DW shoot·plant^{-1} in NO_3-fed APN18 and BAT271, respectively. Under non-limiting P supply, i.e. above 360 P, shoot DW of N_2-dependent plants was less than 50% of that of NO_3-fed plants, except in BAT271 where this ratio reached 80%. This was also observed under limiting P supply, i.e. below 135 P, shoot biomass in N_2-dependent plants being lower than in NO_3-fed plants, except in BAT271 which had a similar shoot DW in both N_2-dependent and NO_3-fed plants (Fig. 1a, b).

Some genotypic differences of root biomass in response to varying P supply were significant for both types of N nutrition (Fig. 1c, d). For N_2-dependent plants, root biomass in BAT271 increased up to 220 P for a maximum of 4.2 g DW root·plant^{-1}, (i.e. the highest among the genotypes, $p < 0.05$), root biomass in APN18 increased up to 135 P for a maximum of 2.8 g DW root·plant^{-1} which was the lowest of all the genotypes ($p < 0.05$). Regardless of P supply, root biomass in G12168 increased up to 135 P for a maximum of

3.2 g DW root·plant^{-1}, which was intermediate between the other genotypes (Fig. 1c).

With NO_3, critical P supplies and maximum value for root biomass were always higher than those observed with N_2-dependent plants, except for G12168 (Fig. 1c, d). Conversely, below 135 P, root biomass was always higher in N_2-dependent than NO_3–N fed plants. Furthermore, root biomass of NO_3-fed plants did not differ between genotypes at 45 P and 72 P, and was higher in BAT271 only at 135 P (Fig. 1d).

The responses of nodule dry weight to varying P supply differed only above 220 P for APN18 compared with BAT271 and G12168 (Fig. 1e). Nodule dry weight increased up to 360 P and 540 P in BAT271 and G12168, respectively. It reached a maximum close to 1.21 g DW nodule·plant^{-1} for both genotypes. Nodule dry weight of APN18 increased similarly to the other genotypes up to 135 P and then more moderately up to 540 P for a maximum of only 0.85 g DW nodule·plant^{-1}. There was no nodule with the NO_3 treatment.

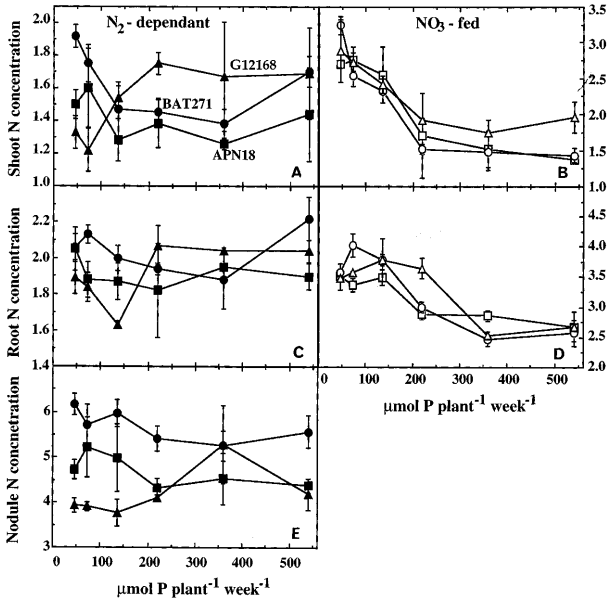

Figure 2. Effect of P supplies on shoot (a, b), root (c, d) and nodule (e) N concentration (percentage, i.e. g N/100 g DW) in bean genotypes (BAT271, G12168, APN18) grown with NO_3 (opened symbols) or with N_2 fixation (closed symbols). Data are the means ± SE of 3 replicated plants, harvested at 50 DAS.

3.2. N concentration, total N accumulation and N use efficiency

Genotypic differences for shoot N concentration in response to varying P supply were significant only for N_2-dependent plants (Fig. 2a). The minimum of ca. 1.4% was observed at 135 P in BAT271 and APN18. By contrast, shoot N concentration increased up to a maximum of 1.7% at 220 P in G12168 (Fig. 2a), which was higher than that of BAT271 ($p < 0.05$). For NO_3-fed plants shoot N concentration decreased up to 220 P for all genotypes, and was slightly higher in G12168 (Fig. 2b).

There was less genotypic difference for N concentration in roots (Fig. 2c, d) than in shoots. For N_2-dependent plants, root N concentration showed no clear pattern of response to varying P supply. The values were in the range of 1.8–2.2%, and differed between genotypes depending on P supply (Fig. 2c). For NO_3-fed plants root N concentration showed a slight increase up to 72 P for BAT271, for a maximum close to 4.0%, and up to 135 P for APN18 and G12168, for a maximum of 3.5 and 3.7%, respectively, and then decreased to reach minimum values close to 2.6%, for the three genotypes. Root N concentrations were higher in NO_3-fed than in N_2-dependent plants regardless of P supply (Fig. 2c, d).

Genotypic differences for nodule N concentration in response to varying P supply were significant. Nodule N concentration slightly decreased with increasing P supply with a mean value of 4.5% in APN18 and 5.5% in BAT271, the latter being the highest among the genotypes, regardless of P supply (Fig. 2e). This parameter in G12168 was the lowest among the genotypes ($p < 0.05$) except at 360 P.

Genotypic differences for total plant N accumulation in response to varying P supply were significant only for N_2-dependent plants (Fig. 3a). Thus, the amount of N-fixed by BAT271 was three-fold higher than that of G12168 (Fig. 3a) at 72 P. It increased up to a maximum of 144 mg N-fixed per plant at 135 P in APN18, though in BAT271 it increased up to 360 mg N-fixed at 540 P, which was the highest ($p < 0.05$) among the genotypes, critical P supply being close to 250 mmol P·plant^{-1}·week^{-1}. N_2 fixation in G12168 was generally intermediate between APN18 and BAT271.

With NO_3, critical P supply for N accumulation was similar to that for N_2-dependent plants in G12168, higher ($p < 0.05$) than that for N_2-dependent plants in APN18 and BAT271. Maximum N accumulation was always higher in NO_3-fed than in N_2-dependent plants and varied between 446 and 512 mg N in G12168 and BAT271, respectively. Under limiting P supply, N accumulation was also higher in NO_3-fed than in N_2-dependent plants, except in BAT271 where N accumulation was similar for both types of N nutrition at 72 P (Fig. 3b).

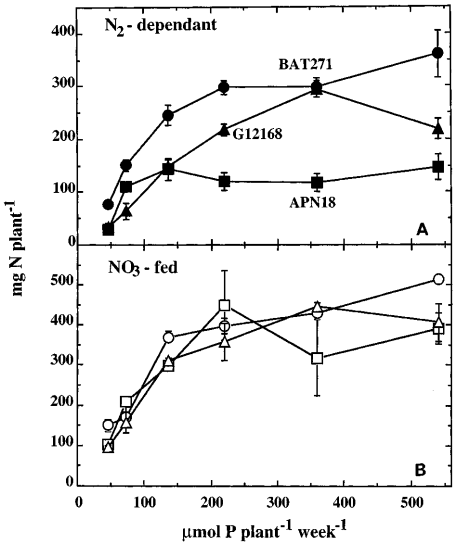

Figure 3. Effect of P supplies on N accumulation in bean genotypes (BAT271, G12168, APN18) grown with NO_3 (opened symbols) or with N_2 fixation (closed symbols). Data are the means ± SE of 3 replicated plants, harvested at 50 DAS.

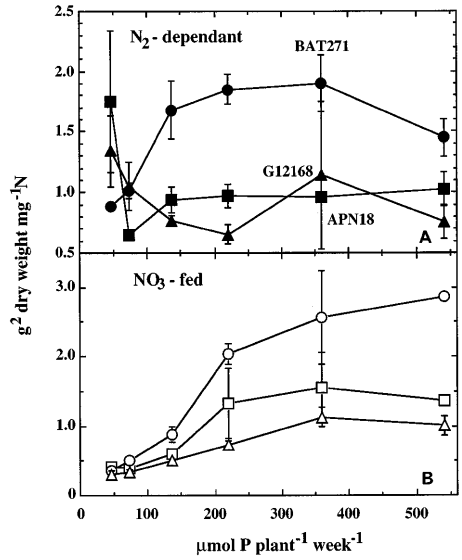

Figure 4. Effect of P supplies on the ratio "biomass/plant N concentration", in bean genotypes (BAT271, G12168, APN18) grown with NO_3 (opened symbols) or with N_2 fixation (closed symbols). Data are the means ± SE of 3 replicated plants, harvested at 50 DAS.

Genotypic differences in response to varying P supply for N use efficiency (NUE), i.e. the ratio biomass:plant N concentration, were significant for both types of N nutrition, although patterns differed greatly between both types of N nutrition for each genotype (Fig. 4a, b). Under limiting P supply, there were no NUE differences between genotypes and, interestingly, NUE was higher ($p < 0.05$) for N_2-dependent than for NO_3-fed plants. In N_2-dependent plants, NUE increased up to 360 P in BAT271 and reached the value of 1.7 compared to less than 1.0 ($p < 0.05$) for other genotypes, while that for APN18 increased up to 135 P only. The critical P supply for NUE was always higher for NO_3-fed than for N_2-dependent plants.

3.3. P concentration and P partitioning

Genotypic differences for shoot P concentration in response to varying P supply were observed for both types of N nutrition, although variation appeared greater for N_2-dependent than for NO_3-fed plants (Fig. 5a, b). For N_2-dependent plants, shoot P concentration increased up to 540 P, except for APN18 which exhibited a decrease between 360 P and 540 P. No critical P supply could be found for any of the three genotypes. Shoot P concentration was the lowest in BAT271, except

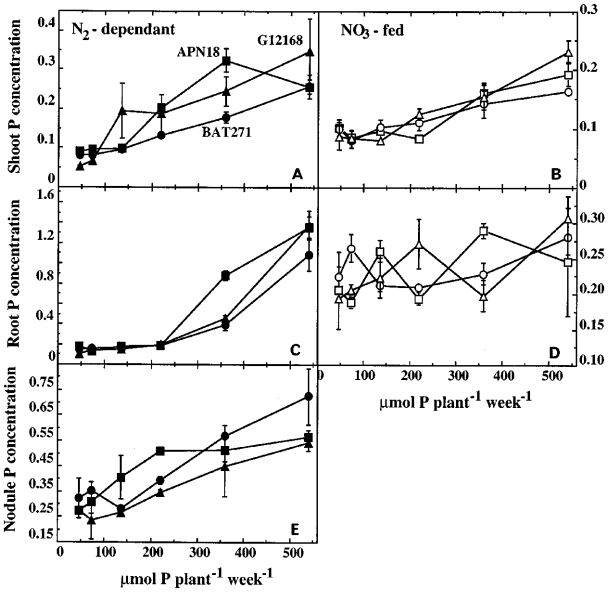

Figure 5. Effect of P supplies on the shoot (a, b), root (c, d) and nodule (e) P concentration (percentage, i.e. g P/100 g DW) in bean genotypes (BAT271, G12168, APN18) grown with NO_3 (opened symbols) or with N_2 fixation (closed symbols). Data are the means ± SE of 3 replicated plants, harvested at 50 DAS.

for P supply below 72 P (Fig. 5a). For NO_3-fed plants, no critical P supply could be found either for shoot P concentration, which also increased up to 540 P. Under non limiting P supply, shoot P concentration values were lower than those of N_2-dependent plants. By contrast, under limiting P supply, shoot P concentration did not differ significantly from one type of N nutrition to another (Fig. 5b).

Response of root P concentration to varying P supply showed no clear pattern for NO_3-fed plants, and values were all in the range of 0.17–0.30% (Fig. 4b). For N_2-dependent plants, the response of root P concentration to varying P supply was similar in all three genotypes (Fig. 4a). The values remained close to 0.12% for P supplies below 220 P, then increased up to values close to 1% (Fig. 5c). Interestingly, root P concentration at P supply below 220 P in NO_3-fed plants was higher than that in N_2-dependent plants (Fig. 5d).

Genotypic differences for nodule P concentration in response to varying P supply were found. Nodule P concentration increased up to 540 P in all three genotypes and no critical P supply was found. For P treatment below 72 P, nodule P concentration was highest in BAT271 and lowest in G12168 (Fig. 5e). For P supply lower than 220 P, the P concentration of nodules was higher than that in other plant tissues.

Genotypic differences of total shoot P in response to varying P supply were found only in N_2-dependent plants (Fig. 6a, b). For these plants, shoot P increased over the whole range of P supply for all genotypes, except for APN18 which did not show any increase beyond 360 P. Except for this genotype, no critical P supply could be found. The highest values for total shoot P were found in G12168. Under limiting P supply there was no difference between genotypes (Fig. 6a). For NO_3-fed plants, total shoot P increased in a similar pattern for all genotypes, and no critical P supply could be found for any of the three genotypes (Fig. 6b).

Genotypic differences of total root P in response to varying P supply were found only in NO_3-fed plants (Fig. 6c, d). For N_2-dependent plants, total root P increased slightly up to 220 P for all genotypes and increased dramatically at higher P supply, showing almost no genotypic differences (Fig. 6c). For NO_3-fed plants, total root P increased in BAT271, reaching no critical P supply. By contrast, total root P was similar for APN18 and G12168 and did not show any significant increase beyond 220 P. Under limiting P supply, total root P was similar for both types of N nutrition, whereas much higher root P was found in N_2-dependent plants relative to NO_3-fed plants at P supplies above 220 P (Fig. 6d).

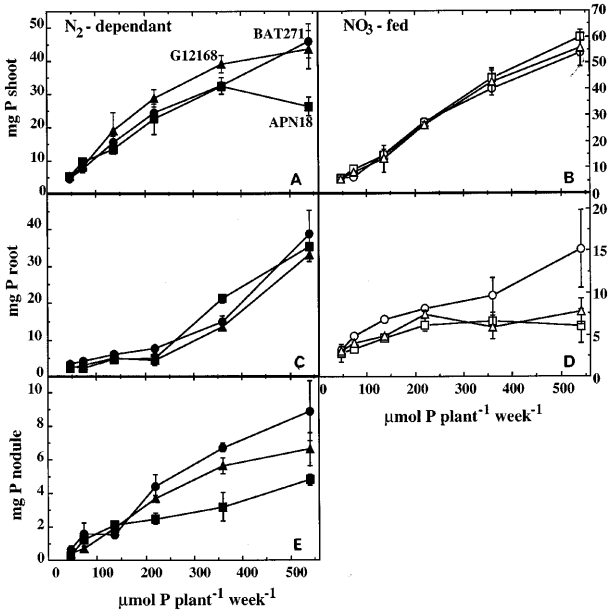

Figure 6. Effect of P supplies on the shoot (a, b), root (c, d) and nodule (e) P content (mg P) in bean genotypes (BAT271, G12168, APN18) grown with NO_3 (opened symbols) or with N_2 fixation (closed symbols). Data are the means ± SE of 3 replicated plants, harvested at 50 DAS.

Genotypic differences of total nodule P in response to varying P supply were significant for P supplies above 135 P. The amount of P in the nodules increased up to 540 P for all genotypes, without reaching any maximum, BAT271 achieving the highest values among the three genotypes (Fig. 6e).

3.4. P use efficiency and P utilization quotient

Genotypic differences for PUE, i.e. the ratio biomass:plant P concentration, in response to varying P supply were significant with both types of N nutrition (Fig. 7a, b). For N_2-dependent plants, PUE increased drastically up to 135 P in BAT271 and then sharply decreased. Values of PUE for BAT271 were the highest among the three genotypes, regardless of P supply. Similarly, PUE in APN18 moderately increased up to 135 P and then decreased up to 540 P. PUE in G12168 moderately decreased from 45 P up to 540 P (Fig. 7a). For NO_3-fed plants, critical P supply for PUE and maximum PUE values were higher than those obtained for N_2-dependent plants. Interestingly, for P supply below

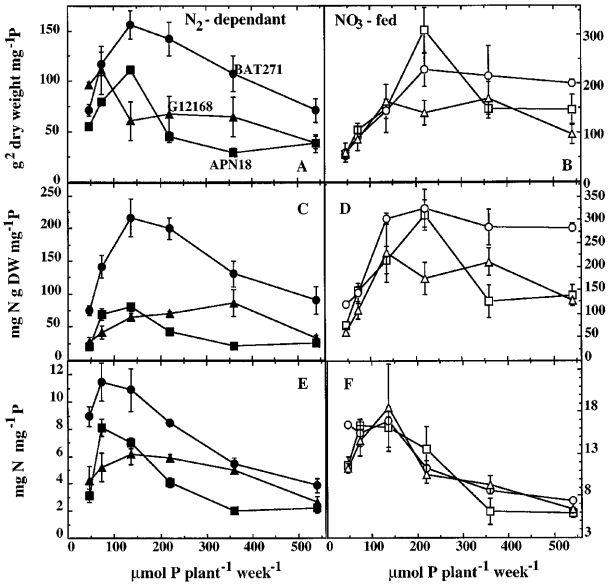

Figure 7. Effect of P supplies on the ratios biomass:plant P concentration (a, b), N accumulation:plant P concentration (c, d), and N accumulation:P accumulation (e, f), in bean genotypes (BAT271, G12168, APN18) grown with NO_3 (opened symbols) or with N_2 fixation (closed symbols). Data are the means ± SE of 3 replicated plants, harvested at 50 DAS.

135 P, PUE values of NO_3-fed plants were lower than those for N_2-dependent plants, with the highest differences for BAT271 (Fig. 7b).

The ratio N accumulation:plant P concentration showed significant genotypic differences in all range of P supplies for N_2-dependent plants, while NO_3-fed plants showed genotypic differences only above 360 P (Fig. 7a, b). For N_2-dependent plants, this ratio increased up to 135 P for BAT271 which reached the highest values among the genotypes, regardless of P supply. It increased up to 135 P in APN18 and up to 360 P for G12168. Therefore, the ratio N accumulation:plant P concentration was higher in APN18 than in G12168 at P supply below 135 P while it was higher in G12168 than in APN18 at P supply above 220 P (Fig. 7a). For NO_3-fed plants, critical P supply for the ratio was higher than for N_2-dependent plants, except for G12168, and maximum values were also higher than for N_2-dependent plants. This ratio increased up to 220 P for BAT271 and APN18 while it increased up to 135 P for G12168. At P supply below 220 P, this ratio did not vary between genotypes (Fig. 7b).

The ratio N accumulation:P accumulation, or N:P, was compared to the ratio N accumulation:plant P concentration. Genotypic differences of the ratio N:P in response to varying P supply were significant for N_2-dependent plants only (Fig. 8a, b). This ratio increased up to 72 P for APN18 and BAT271, the latter having the highest values among the genotypes, regardless of P supply, while it increased up to 135 P for G12168 (Fig. 7a). For NO_3-fed plants, critical P supply for the ratio N:P was similar to that of N_2-dependent plants, except for BAT271. Maximum values for this ratio were also higher than for N_2-dependent plants. No genotypic differences were however recorded (Fig. 7b).

4. DISCUSSION

Our work shows genotypic variation in P requirement for N_2 fixation in common beans (Fig. 3). This P requirement was either similar to (BAT271, G12168) or lower than (APN18) that for NO_3-fed plants. This contrasts with soybean for which P requirement for maximum N accumulation was higher for N_2-dependent than for NO_3-fed plants [17], although in cowpea, similar P requirement was found for N_2-dependent and NO_3-fed plants [4]. However, the previous conclusions that P requirement for the N_2-fixation metabolism is higher than that for shoot growth [4, 12, 16, 28] is, in our work with common bean, supported by the genotypic differences in N accumulation as a function of P that were found only in N_2-dependent, and not NO_3-fed, plants (Fig. 3).

The relation we have found between genotypic differences in P requirement for N_2 fixation and PUE (biomass:plant P concentration) (Fig. 7a, b) agrees with previous similar conclusions in soybean [12] and *Acacia mangium* [32]. However in the present work, the ratio N accumulation:plant P concentration (PUEN) (Fig. 7c, d) and N:P (Fig. 7e, f), were more correlated to genotypic differences in N accumulation for N_2-fixing common bean plants than the ratio PUE especially with P supplies lower than 220 P. Though, the ratio N:P did not differ between genotypes for NO_3-fed plants in contrast with PUE or PUEN (Fig. 7). We attribute this contrast to differences in nitrogen use efficiency (biomass:plant N concentration, NUE) between genotypes especially for plants relying on N_2 fixation (Fig. 4). Thus, the NUE affects the PUE and PUEN through its effect on plant DW. Though, the NUE does not affect the ratio N:P which is independent of plant DW. For example, a larger difference was found between BAT271 and APN18 at 72 P with PUEN than with N:P (Fig. 7). Thus, BAT271 was significantly more efficient than APN18 in the use of P for N_2 fixation. This was due to a NUE of 1 for BAT271 versus 0.5 for APN18, this difference being

higher for N_2-dependent than for NO_3-fed plants, especially because of higher NUE for N_2-dependent plants of BAT271. Therefore, the higher PUE of BAT271 was not only due to its higher efficiency in the use of P for N_2 fixation, but also to its higher efficiency to use the N-fixed for growth (NUE). This leads us to recommend the ratio PUEN as a better screen than the ratio N:P or simply plant N accumulation, for tolerance of N_2 fixation to P deficiency. Indeed, when considering only the N accumulation (Fig. 3) and the ratio N:P (Fig. 7) at 72 P, APN18 might have been chosen as a P tolerant N_2-dependent genotype like BAT271 by contrast with G12168. Under N_2-fixation, growth of APN18 was comparable to that of G12168, i.e. significantly lower than that of BAT271 (Fig. 1), as confirmed by close values for PUEN of APN18 and G12168, which were significantly lower than that of BAT271 (Fig. 7).

The higher amount of N-fixed with lower or similar nodule P concentration below 220 P for BAT271 compared with APN18 or G12168 below 220 P suggests a more efficient use of P in the N_2-fixing metabolism of BAT271 nodules. This is further substantiated by a similar nodule mass for the 3 genotypes, at least below 135 P. The latter agrees with previous observations of the absence of correlation between N_2 fixation capacity and nodule mass under P deficiency [12, 33]. Moreover, in BAT271 a large part of the N requirement could be met by N_2 fixation, like in a few other common bean genotypes [20, 26]. Indeed, at 50 DAS, the amount of N-fixed by BAT271 was close to the amount of N accumulated by the NO_3-fed plants, especially under P deficiency, and additional amounts of N were likely to be fixed during subsequent growth [3, 35].

Most common bean genotypes have however low N_2-fixing capacity [10, 15, 24, 25], possibly because common bean was originally domesticated as a home-garden crop receiving anthropic organic residues resulting in low selection pressure on the symbiosis with rhizobia (Debouck, pers. comm.), and more recently bred without reference to improving N_2 fixation per se [21]. Therefore, our demonstration of genotypic variability in PUE for N_2 fixation in common bean suggests that besides selecting highly nodulating lines [19], the improvement of N_2 fixation might also be reached by using PUEN at 72 and 360 μmol $P \cdot plant^{-1} \cdot week^{-1}$ in a broader genotypic screening in our liquid-culture system. However, subsequent comparison of contrasting genotypes will be needed in soil. More work is also required for further understanding of the partitioning and use of P in nodules. Although it is often favored to breed for increased P acquisition [1, 9, 11], increased PUE in a N_2-fixing legume may be more sustainable in terms of soil P resources and cropping system productivity.

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REFERENCES

[1] Adu-Gyamfi J.J., Fujita K., Ogata S., Phosphorous absorption and utilization efficiency of pigeon pea (*Cajanus Cajan* L. Millsp.) in relation to dry matter production and dinitrogen fixation, Plant and Soil 119 (1989) 315–324.

[2] Anuradha M., Narayanan A., Promotion of root elongation by phosphorous deficiency, Plant and Soil 36 (1991) 273–275.

[3] Attewell J., Bliss F.A., Host plant characteristics of common bean lines selected using indirect measures of N_2 fixation, in: Evans H.J., Bottomley P., Newton W.R. (Eds.), Nitrogen Fixation Research Progress, Martinus Nijhoff, Dordrecht, 1985, pp. 3–9.

[4] Cassman K.G., Whitney A.S., Fox R.L., Phosphorous requirements of soybean and cowpea as affected by mode of N nutrition, Agron. J. 73 (1981) 17–22.

[5] Clarkson D.T., Factors affecting mineral nutrient acquisition by plants, Annu. Rev. Plant Physiol. 36 (1985) 77–115.

[6] Darrah P.R., The rhizosphere plant nutrition: a quantitative approach, Plant and Soil 155/156 (1993) 1–20.

[7] Drevon J.J., Kalia V.P., Heckmann M.O., Pédelahore P., In situ open–flow assay of acetylene reduction activity by soybean root nodules: Influence of acetylene and oxygen, Plant Physiol. Biochem. 26 (1988) 73–78.

[8] Fohse D., Claassen N., Jungk A., Phosphorous efficiency of plants I: External and internal P requirement and P uptake efficiency of different plant species, Plant and Soil 110 (1988) 101–109.

[9] Gourley C.J.P., Allan D.L., Russelle M.P., Defining phosphorous efficiency in plants, Plant and Soil 155–156 (1993) 289–292.

[10] Graham P.H., Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris*, Field Crop Res. 4 (1981) 93–112.

[11] Gunawardena S.F.B.N., Danso S.K.A., Zapata F., Phosphorous requirement and sources of nitrogen in three soybean (*Glycine max*) genotypes: Bragg, nts 382 and Chippewa, Plant and Soil 151 (1993) 19–26.

[12] Gunawardena S.F.B.N., Danso S.K.A., Zapata F., Phosphorous requirement and nitrogen accumulation by three mungbean (*Vigna radiata* (L.) Welzek) cultivars, Plant and Soil 147 (1992) 267–274.

[13] Hawkesford M.J., Belcher A.R., Differential protein synthesis in response to sulphate and phosphate deprivation, Planta 185 (1991) 323–329.

[14] Hinsinger P., Structure and function of the rhizosphere: mechanisms at the soil-root interface, OCL, Oleagineux Corps Gras Lipides 5 (1998) 340–341.

[15] Isoi T., Yoshida S., Low nitrogen fixation of common bean (*Phaseolus vulgaris*), Soil Sci. Plant Nutr. 37 (1991) 559–563.

[16] Israel D.W., Investigation of the role of phosphorous in symbiotic dinitrogen fixation, Plant Physiol. 84 (1987) 835–840.

[17] Israel D.W., Rufty T.W. Jr., Influence of phosphorous nutrition on phosphorous and nitrogen utilization efficiencies and associated physiological responses in soybean, Crop Sci. 28 (1988) 954–960.

[18] Jakobsen I., The role of phosphorous in nitrogen fixation by young pea plants (*Pisum sativum*), Physiol. Plant. 64 (1985) 190–196.

[19] Kipe-Nolt J.A., Vargas H., Giller K.E., Nitrogen fixation in breeding lines of *Phaseolus vulgaris* L., Plant and Soil 152 (1993) 103–106.

[20] Kumarasinghe K.S., Danso S.K.A., Zapata F., Field evaluation of N_2 fixation and N partitionning in climbing bean (*Phaseolus vulgaris* L.) using ^{15}N, Biol. Fert. Soils 13 (1992) 142–146.

[21] Miranda B.D., Bliss F.A., Selection for increased seed nitrogen accumulation in common bean: Implications for improving dinitrogen fixation and seed yield, Plant Breed. Rev. 106 (1991) 301–311.

[22] Murphy J., Riley J.P., A modified single solution method for the determination of phosphate in natural waters, Anal. Chem. Acta 27 (1962) 31–36.

[23] Ohwaki Y., Hirata H., Differences in carboxylic acid exudation among P-starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid levels in roots, Soil Sci. Plant Nutr. 38 (1992) 235–243.

[24] Pereira P.A.A., Bliss F.A., Nitrogen fixation and plant growth of common bean (*Phaseolus vulgaris* L.) at different levels of phosphorous availaibility, Plant and Soil 104 (1987) 79–84.

[25] Pereira P.A.A., Bliss F.A., Selection of common bean (*Phaseolus vulgaris* L.) for N_2 fixation at different levels of available phosphorous under field and environmentally-controlled conditions, Plant and Soil 115 (1989) 75–82.

[26] Rennie R.J., Kemp G.A., ^{15}N-determined time course of N_2 fixation in two cultivars of field bean, Agron. J. 76 (1984) 146–154.

[27] Ribet J., Drevon J.J., The phosphorous requirement of N_2-fixing and urea-fed *Acacia mangium*, New Phytol. 132 (1997) 383–390.

[28] Sa T.M., Israël D.W., Energy status and functioning of phosphorous deficient soybean nodules, Plant Physiol. 97 (1991) 928–935.

[29] Sanginga N., Danso S.K.A., Bowen G.D., Nodulation and growth response of *Allocasuarina* and *Casuarina* species to phosphorous fertilization, Plant and Soil 118 (1989) 125–132.

[30] Siddiqi M.M., Glass A.D.M., Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants, J. Plant Nutr. 4 (1981) 289–302.

[31] Sun J.S., Simpson R.J., Sands R., Nitrogenase activity and associated budgets in seedlings of *Acacia mangium* measured with a flow-through system of the acetylene reduction assay, Austr. J. Plant Physiol. 19 (1992) 97–107.

[32] Vadez V., Lim G., Durand P., Diem H.G., Comparative growth and symbiotic performance of four *Acacia mangium* provenances from Papua New Guinea in response to the supply of phosphorous at various concentrations, Biol. Fertil. Soils 19 (1995) 60–64.

[33] Vadez V., Variabilité génétique de la fixation d'azote sous carence en phosphore chez le haricot : relations avec l'efficacité d'utilisation du P et la perméabilité nodulaire à l'oxygène, thèse de Doctorat, Montpellier, France, 1996, 150 p.

[34] Vadez V., Beck D.P., Drevon J.J., Utilization of the acetylene reduction assay to screen for tolerance of symbiotic N_2 fixation to limiting P nutrition in common bean, Physiol. Plant. 99 (1997) 227–232.

[35] Wolyn D.J., Attewell J., Ludden P.W., Bliss F.A., Indirect measures of N_2 fixation in common bean (*Phaseolus vulgaris* L.) under field conditions: The role of lateral root nodules, Plant and Soil 113 (1989) 181–187.

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