

Symbiotic response to low phosphorus supply in two common bean (*Phaseolus vulgaris* L.) genotypes

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(Received October 30, 2006; Accepted April 11, 2007)

Abstract

The nodulation, N₂ fixation and growth of two bean (*Phaseolus vulgaris* L.) genotypes (cvs. Alubiacerillos and Flamingo) were studied in relation to P supplies (75 versus 250 μmol P plant⁻¹ week⁻¹) in nutrient solution. The two cultivars were inoculated separately with three *Rhizobium* strains (Ar2, CIAT899 and KH28). The effect of low P on nodulation, nitrogen accumulation and growth varied with both the bean cultivar and the *Rhizobium* strain. The cv. Flamingo was less susceptible to low P than cv. Alubiacerillos. The decrease in assessed parameters was lower with KH28 than with the remaining strains. Overall, plants inoculated with KH28 were the most efficient with respect to the rhizobial symbiosis. There is thus, potential for improving tolerance to low P availability in beans through effective exploitation of this symbiosis.

Keywords: Symbiosis, *Phaseolus vulgaris*, rhizobia, phosphorus

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for human consumption worldwide, especially in Latin America and Africa (CIAT, 1992). Its cultivation in these regions extends into marginal areas where P deficiency is one of the major yield-limiting factors for plants (CIAT, 1992). P has a key role in the energy metabolism of all plant cells, and particularly in nitrogen fixation as an energy-requiring process (Dilworth, 1974). Sa and Israel (1991) reported that P deficiency has a negative impact on the energy status of legume nodules. P requirements for symbiotic nitrogen fixation were found to vary with genotypes in *Cajanus cajan* (Adu et al., 1989) and were related to P use efficiency in genotypes of Soya (Gunawardena et al., 1993). P use efficiency in nitrogen-fixing symbioses is influenced by an adequate distribution

of the P between the shoots and nodulated roots and between roots and nodules (Cassman, 1981).

Nodule number and mass, as well as total and specific nitrogenase activity, decrease with low P supplies (Israel, 1987; Ribet and Drevon, 1995; Drevon and Hartwig, 1997). Studies with common bean relate P limitation to phosphate-use efficiency (Vadez et al., 1999), carbon economy (Nielsen et al., 2001), photosynthetic activity (Liao and Yan, 1999) and the assimilation and translocation of nitrates (Gniazdowska et al., 1999). Phosphatase activities of intact roots nodules may have a significant role in making ion-available P more available for plant use (Bieleski, 1973). Thus, effective exploitation of rhizobial symbiosis may significantly improve tolerance to low P availability in legumes.

The aim of the present work was to compare the response, at low P supply, of two common bean cultivars in symbiotic association with three *Rhizobium* strains.

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2. Material and Methods

Biological material, culture conditions and harvest

Two common bean cultivars, the white seed Alubiacerillos and the brown seed Flamingo were used and inoculated with one of three *Rhizobium* strains: *Rhizobium tropici* CIAT899, a local *Rhizobium* strain, Ar2, isolated in the northern Tunisia and KH28 from France. Seeds of the above cultivars were surface sterilized (calcium hypochlorite (2%) 5–7 minutes), and then washed with sterile, distilled water (30 min). The seeds were pre-germinated in agar 0.9% for 3–4 days. *Rhizobium* inoculation was done with a liquid culture in YEM medium (Vincent, 1970), which was applied by soaking the seedlings in the inoculants prior to transplantation. The seedlings were then transferred in hydroponic culture system consisting of containers filled with 45 l of nutrient solution and 20 seedlings were inserted in each container (providing about 2.25 l of nutrient solution per seedling). The roots of the selected, uniform, seedlings were passed through the hole of a rubber stopper on the container cover, and a cotton wool was fitted at the hypocotyls level to maintain the root system suspended in the nutrient solution (Vadez et al., 1996).

P was supplied with 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ versus 250 $\mu\text{mol P}$ as control. During the first 2 weeks, i.e. before nodule function, the nutrient solution was supplemented with 2 mM urea. Thereafter, the nutrient solution, without urea, was renewed every 2 weeks and to adjust the pH, 0.2 g of calcium carbonate were added for every litre of the 45 l nutrient solution in a container. It was aerated with a flow of 400 ml min^{-1} of filtered air.

Plants were grown in a temperature-controlled glass-house with night/day temperatures of circa 20/28°C and a 16 h photoperiod with additional lights of 400 $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$. Plants were harvested at late flowering (R7). Shoots and roots were separated and the nodules were removed and counted. Shoots, roots and nodules were dried in an oven at 70°C to determine their dry weights and to enable calculation of the root to shoot ratio (RSR). Samples of shoots were ground and nitrogen content was measured by the Kjeldahl procedure. Seven replicates were used to calculate the mean of each parameter for each treatment. The efficiency in utilization of the rhizobial symbiosis (EURS) was estimated by the slope of the regression model of plant biomass as a function of nodule biomass. For a linear adjustment-curve, i.e. $y = ax + b$, b corresponds to the plant biomass production without nodules (g pDW₀), and a corresponds to the EURS as (g pDW – g pDW₀) g^{-1} nDW.

Data analysis

The analysis of data was performed using the SAS

system for windows V8 software. Means were separated by Fisher's LSD-test.

3. Results

Symbiotic parameters

The effect of low P supply on symbiotic parameters (nodule number and nodule dry weight) was analysed in Flamingo and Alubiacerillos inoculated with three *Rhizobium* strains (Table 1). The infectivity potential of the inoculated strain varied with the cultivar.

In the plants of cv. Alubiacerillos, supplied with 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$, strain KH28 gave the highest infectivity 680 nodules per plant. In contrast, plants inoculated with CIAT899 exhibited the lowest nodule number (59 nodules plant^{-1}). However, the highest total nodule dry weight and highest dry weight per nodule was recorded with Ar2 (776 mg plant^{-1}) and with CIAT899 (10 mg nodule^{-1}), respectively. In plants, supplied with 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$, inoculation with KH28 led to significantly higher number of nodules and total nodule mass than with the remaining rhizobia strains. P-deficiency, 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$, caused a large reduction in nodule biomass in plants infected with Ar2 (91%), a lesser decrease with CIAT899 (77%), and the least was recorded with KH28 (49%).

In cv Flamingo, plants supplied with 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ did not show a significant variation in nodule number (Table 1). On the other hand, total nodule mass was affected by the *Rhizobium*, being significantly higher with CIAT899 (752 mg plant^{-1}) and Ar2 (733 mg plant^{-1}) than with KH28 (526 mg plant^{-1}). P-deficiency did not significantly affect nodule number with CIAT899 and Ar2. However, there were significantly more nodules with strain KH28 in plants supplied with 75 $\mu\text{mol P}$ than those given 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$. Indeed, the number of nodules induced by inoculation with KH28, at 75 $\mu\text{mol P}$, was more than twice that observed with the remaining strains. Nodule dry weight was significantly decreased at 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ but plants inoculated with CIAT899 exhibited the highest nodule dry weight.

Growth parameters

Plant growth in terms of shoot dry weight, root dry weight and root to shoot ratio (RSR) showed a variation depending on the P supply and the rhizobia (Table 2). In Alubiacerillos, under 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ conditions, shoot dry weight was similar regardless of which *Rhizobium* strain was used, ranging from 6.3 to 6.8 g plant^{-1} (Table 2). P-deficiency at 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$, significantly reduced shoot dry weight, but the decrease was significantly greater with CIAT899 (48%) than with

Table 1. Variation of symbiotic parameters (nodule number and nodule mass) in two common bean cultivars (Alubiaccerrillos and Flamingo) inoculated with three *Rhizobium* strains (CIAT899, Ar2 and KH28) under 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ and 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ conditions in glasshouse. Plants were harvested at R7.

<i>Rhizobium</i>	Treatment	Nodule number per plant		Nodule dry weight (g plant ⁻¹)	
		Alubiaccerrillos	Flamingo	Alubiaccerrillos	Flamingo
CIAT899	250 $\mu\text{M P}$	59	198	0.590	0.752
	75 $\mu\text{M P}$	128	125	0.137	0.635
Ar2	250 $\mu\text{M P}$	156	189	0.776	0.733
	75 $\mu\text{M P}$	103	138	0.067	0.343
KH28	250 $\mu\text{M P}$	680	200	0.522	0.526
	75 $\mu\text{M P}$	516	305	0.266	0.302
C.V. (%)		34.17	38.48	30.51	13.32
L.S.D.		83	77	0.111	0.072

Table 2. Variation of growth parameters (shoot dry weight, root dry weight and root to shoot ratio) in two common bean cultivars (Alubiaccerrillos and Flamingo) inoculated with three *Rhizobium* strains (CIAT899, Ar2 and KH28) under 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ and 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ conditions in glasshouse. Plants were harvested at R7.

<i>Rhizobium</i>	Treatment	Shoot dry weight (g plant ⁻¹)		Root dry weight (g plant ⁻¹)		Root to shoot ratio (RSR)	
		Alubiaccerrillos	Flamingo	Alubiaccerrillos	Flamingo	Alubiaccerrillos	Flamingo
CIAT899	250 $\mu\text{M P}$	6.750	8.389	1.107	1.463	0.170	0.176
	75 $\mu\text{M P}$	3.529	6.114	1.233	1.132	0.357	0.189
Ar2	250 $\mu\text{M P}$	6.801	8.623	1.487	0.982	0.219	0.115
	75 $\mu\text{M P}$	5.527	7.494	1.570	1.235	0.289	0.165
KH28	250 $\mu\text{M P}$	6.294	8.266	1.123	1.157	0.180	0.141
	75 $\mu\text{M P}$	5.510	7.214	1.703	2.237	0.319	0.317
C.V. (%)		13.28	7.84	31.29	26.62	40.13	29.45
L.S.D.		0.769	0.647	0.449	0.388	0.116	0.057

Table 3. Variation of efficiency in utilization of the rhizobial symbiosis (EURS) in two common bean cultivars (Alubiaccerrillos and Flamingo) inoculated with three *Rhizobium* strains (CIAT899, Ar2 and KH28) under 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ and 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ conditions. Plants were harvested at R7.

Cultivar	Treatment	CIAT899	Ar2	KH28
Alubiaccerrillos	250 $\mu\text{M P}$	11.52	1.7	9.75
	75 $\mu\text{M P}$	-9.59	-10.86	9.70
Flamingo	250 $\mu\text{M P}$	8.51	6.14	5.40
	75 $\mu\text{M P}$	1.24	-1.06	8.75

Ar2 (19%) and KH28 (12%). Root dry weight did not vary significantly under 75 $\mu\text{mol P}$ with any of the strains except with KH28 which showed significant increase (52%) in root weight. Root to shoot ratio was not variable with rhizobia in control plants, but increased significantly with CIAT899 and KH28 under P-deficient conditions.

In Flamingo, the shoot dry weight was higher than that recorded in Alubiaccerrillos for all three *Rhizobium* strains. The shoot dry weight was not significantly different in plants supplied with 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$, whatever

strain was used. Plants given 75 $\mu\text{mol P}$ showed a significant decrease of shoot dry weight with all *Rhizobium* strains; the decrease was higher with CIAT899 (27%) than with the remaining rhizobia (13%). With 250 $\mu\text{mol P}$, root dry weight was significantly higher with CIAT899 than with Ar2 whereas under 75 $\mu\text{mol P}$, root weight was greatest with KH28 (93%) but not significantly affected with the remaining strains. Root to shoot ratio was significantly higher with CIAT899 than with Ar2 in plants supplied with 250 $\mu\text{mol P}$, whereas it was greatest with KH28 in plants receiving 75 $\mu\text{mol P}$ (Table 2).

Nitrogen fixation

Amount of fixed-N showed important variations depending both on the cultivar and the rhizobia (Fig. 1). In Alubiaccerrillos, under 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$, CIAT899 and to a lesser extent KH28 fixed significantly larger amount of N than Ar2. P deficiency at 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$, greatly reduced N fixation in plants infected with CIAT899 but did not significantly affect plants grown with the remaining strains; KH28 fixed the largest amount of N. In Flamingo, under 250 $\mu\text{mol P}$, CIAT899 and Ar2 fixed

significantly larger amount of N than KH28 (Fig. 1). In plants supplied with 75 $\mu\text{mol P}$, the amount of N fixation was greatly reduced with Ar2 and to a lesser extent with CIAT899 as compared to KH28. All the *Rhizobium* strains fixed less N when grown with cv. Alubiacerillos than with cv. Flamingo.

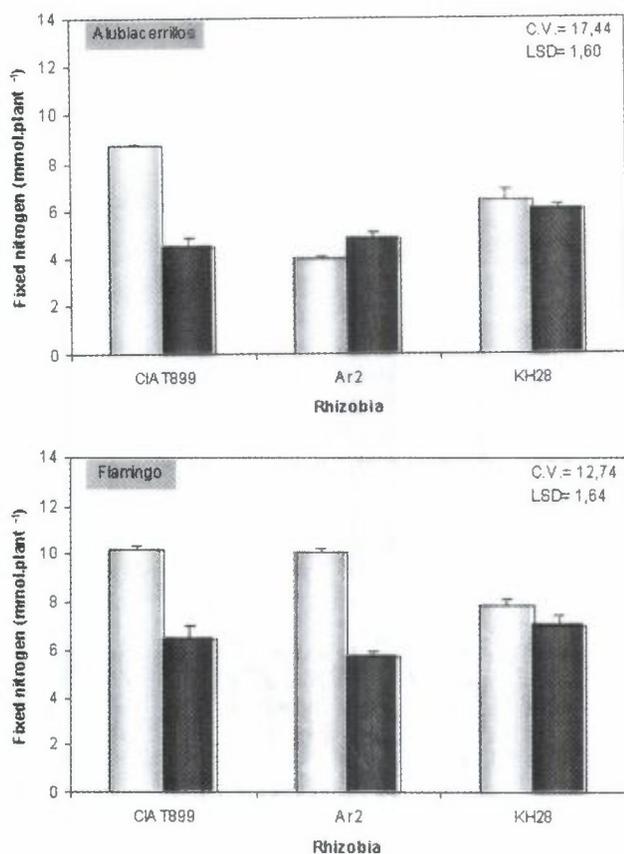


Figure 1. Variation of fixed nitrogen in two common bean cultivars (Alubiacerillos and Flamingo) inoculated with three *Rhizobium* strains (CIAT899, Ar2 and KH28) under 250 $\mu\text{mol P}$ plant⁻¹ week⁻¹ (□) and 75 $\mu\text{mol P}$ plant⁻¹ week⁻¹ (■) conditions in glasshouse. Plants were harvested at R7.

The efficiency in utilization of the rhizobial symbiosis (EURS)

The efficiency in utilization of the rhizobial symbiosis (EURS) estimated by the slope of the linear regression model of plant biomass as a function of nodule dry weight was analysed for the six experimental treatments (Table 3). In Alubiacerillos, nodules were least efficient with Ar2, the EURS being lower than 2 versus higher than 9 with the remaining *Rhizobium* strains. Under 75 $\mu\text{mol P}$, this efficiency parameter showed a great decrease with CIAT899 and Ar2. By contrast, with KH28, the EURS did not vary. In Flamingo, plants given 250 $\mu\text{mol P}$ exhibited the lowest EURS with KH28, and the highest with

CIAT899. At 75 $\mu\text{mol P}$, the EURS was reduced with all the *Rhizobium* strains except KH28 which showed an increase.

4. Discussion

Reports on the effect of P-deficiency on nodule number are still controversial and this deficiency may result in an increase, decrease or even not affect nodule number per unit of shoot biomass (Gunawardena et al., 1992; Ribet and Drevon, 1995; Vadez et al., 1996; Drevon and Hartwig, 1997). In the present work, P-deficiency reduced nodulation; however, in both cultivars nodule number was significantly higher with KH28 than with the remaining strains (Table 1). Besides, P-deficiency significantly increased nodule number as recorded in the symbiotic association "Flamingo-KH28" (Table 1). The best nodulation potential as expressed with nodule dry weight, under P-deficient conditions, was recorded in Flamingo with CIAT899 and in Alubiacerillos with KH28 and CIAT899. The highest total and individual nodule mass (635 mg plant⁻¹ and 5.1 mg nodule⁻¹, respectively) was recorded with CIAT899 in plants receiving 75 $\mu\text{mol P}$ plant⁻¹ week⁻¹ (Table 1). P-deficiency may indirectly affect nodule development, by restricting metabolite supply from the host plant, and has previously been reported to decrease nodule mass more than host growth in soybean (Israel, 1987; Ribet and Drevon, 1995; Drevon and Hartwig, 1997), common bean (Pereira et al., 1989; Vadez et al., 1996), pea (Jacobsen, 1985) and alfalfa (Drevon and Hartwig, 1997). Differences in the response of nodulation to P-deficiency appear to be related to legume species, genotype, *Rhizobium* strain and experimental conditions.

Our data demonstrate a variation in vegetative growth response in relation to cultivars and P-deficiency. Alubiacerillos was less susceptible to P-deficiency at 75 $\mu\text{mol P}$ with KH28 in terms of shoot and root biomass and root to shoot ratio (Table 2). Flamingo exhibited superior vegetative growth under both P-supply regimes. In P-deficient plants at 75 $\mu\text{mol P}$, growth reduction was less than 20% with all the rhizobia except CIAT899 (30%). On the other hand, root dry weight was greatly increased by P-deficiency with KH28 and Ar2, in contrast to CIAT899 (Table 2). It was suggested that the decrease of shoot dry weight in P-deficient plants may be a direct consequence of a reduction of leaf expansion and reduced leaf initiation (Lynch et al., 1991; Liao and Yan, 1999). The reduction of leaf area at low P was found by Chaudhary and Fujita (1998) in several legumes. According to Lynch and Beebe (1995) the reduction of the growth in P-deficient plants is associated with an increase of root mass and a decrease in leaf growth rate (Aiken and Smucker, 1996).

Results on nitrogen fixation showed that the most efficient associations were those with CIAT899 and, to a

lesser extent, with Ar2, when the plants received 250 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$ (Fig. 1). In plants supplied with only 75 $\mu\text{mol P plant}^{-1} \text{ week}^{-1}$, strain KH28, showed the least reduction in fixation for both cultivars. P requirements for symbiotic nitrogen fixation have been found to vary with genotype, particularly in *Cajanus cajan* (Adu et al., 1989). Differences in symbiotic nitrogen fixation in relation to P use efficiency were also reported for Soya genotypes (Gunawardena et al., 1993). The P use efficiency in plants depends upon the distribution of the P between the shoots and nodulated roots and between roots and nodules (Cassman et al., 1981). Bean genotypes differ in their ability to fix nitrogen when the P is limiting (Yan et al., 1995) and this is in agreement with our results (Fig. 1). Under 75 $\mu\text{mol P}$, the cv. Flamingo in association with KH28 showed the largest nitrogen fixation, and cv. Alubiacerillos was also most productive under reduced P conditions with this strain, KH28. Furthermore, with both cultivars, the highest EURS was recorded with KH28 in P-deficient plants (Table 3). This supports the ideas of Liao and Yan (2000), that the capacity to fix nitrogen under P-deficient conditions is related to the P use efficiency. In conclusion, the present study suggests that the effective use of the appropriate *Rhizobium* strain, in the bean symbiosis, may significantly improve tolerance to P-deficiency and could, therefore, be of considerable agronomic value and importance.

Acknowledgements

The authors wish to acknowledge 'La Coopération Franco-Nigérienne' for financial support, Payre Hélène for technical assistance, and Professor D.H.S Richardson for help with the English and editing an early draft of the manuscript.

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