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ORIGINAL PAPER

Relationship between phosphorus status and nitrogen fixation by common beans (*Phaseolus vulgaris* L.) under drip irrigation

H. A. Attar · D. Blavet · E. M. Selim ·
M. T. Abdelhamid · J. J. Drevon

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Abstract The current study aims to examine, the response of contrasted recombinant inbred lines of common bean to the application of phosphorus, to identify the bean recombinant inbred lines which were efficient in phosphorus utilization when dependent on nitrogen fixation as a source of nitrogen. The experiment was conducted at the experimental farm of Agricultural Research Station of the Nubaria district, Behera, Egypt, during the winter seasons of 2008–2009. Three levels of mineral phosphorus fertilizers were applied (0, 45 and 90 kg ha^{-1} phosphorus pentoxide). Nodulation, plant growth parameters, leaf area, soil Olsen phosphorus, pH, and phosphorus and nitrogen of shoots, nodules and seeds were measured. The results have shown that the recombinant inbred lines responded positively to P application levels. The best values were observed in recombinant inbred lines 75, 83 and 34. Vegetative growth parameters were significantly enhanced by increasing levels of phosphorus. The highest level of phosphorus, i.e., 90 kg ha^{-1} phosphorus pentoxide gave the optimal values of growth parameters for all common bean recombinant inbred lines while control plants obtained the lowest values. An increase of Olsen-P and a decrease of soil pH were also observed with increases in phosphorus. These results led to the conclusions that phosphorus applied to Nubaria soil: (1) improved the soil fertility; (2) enhanced the ability of root nodules of common bean recombinant inbred lines to fix atmospheric nitrogen; and (3) increased the release of hydrogen by roots, thus decreasing soil pH and reducing the immobilization of phosphorus in the soil solution and transforming it into available form for the plant.

Keywords Drip irrigation · Nitrogen fixation · Phosphorus · *Phaseolus vulgaris* · Recombinant inbred lines · Sandy soil

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H. A. Attar (✉) · E. M. Selim
Soils and Water Use Department, Agricultural and Biological Division, National Research Centre, 33 Al Behoos St.,
P.O. Box 12622, Dokki, Cairo, Egypt
e-mail: hamzahesham@hotmail.com

E. M. Selim
e-mail: selimscience@gmail.com

H. A. Attar · D. Blavet · J. J. Drevon
INRA-IRD-SUPAGRO, Ecologie Fonctionnelle et Biogéochimie des Sols et des Agro-écosystèmes,
place Pierre Viala, 34060 Montpellier Cedex 1, France

D. Blavet
e-mail: didier.blavet@ird.fr

J. J. Drevon
e-mail: drevonjj@supagro.inra.fr

E. M. Selim
Biological Sciences Department, Rabigh-College of Science & Art, King Abdulaziz University, P.O. Box 344,
Rabigh 21911, Saudi Arabia

M. T. Abdelhamid
Botany Department, Agricultural and Biological Division,
National Research Centre, 33 Al Behoos St.,
P.O. Box 12622, Dokki, Cairo, Egypt
e-mail: magdi.abdelhamid@yahoo.com

Introduction

Phosphorus in the soil is, with nitrogen, a major limiting mineral nutrient for both plant growth and the development of microorganisms (Vance et al. 2003). This is particularly true in newly reclaimed desert soils, and notably in the calcareous and sandy ones, which have low availability of phosphorus and micronutrients for the plants (Koreish et al. 1998). These sandy soils have naturally low contents of nutrients, and low contents of organic matter and clay minerals that prevents the soil from preserving water and nutrients (Abou Hussien et al. 2002). Moreover, the application of mineral fertilizers to these calcareous and sandy soils may be ineffective because of their alkaline reaction (Wahba et al. 2000).

Newly reclaimed desert soils exist in Egypt which is facing a limitation in fertile cultivated soils in the old Nile Valley and Delta, which represent about 3–4% of the total area of Egypt. Thus, for the last several years, special attention has been directed towards reclaiming desert soils, sandy or calcareous in nature. Globally the consumption of N-fertilizer increased from 8 to 17 kg ha^{-1} of agricultural land in the 15 years period from 1973 to 1988 (FAO 1990). Significant growth in fertilizer-N usage has occurred in both developed and developing countries (Peoples et al. 1995). The requirements for fertilizer-N are predicted to increase further in the future (Subba-Rao 1980); however, with the current technology for fertilizer production and the inefficient methods employed for fertilizer application, both the economic and ecological costs of fertilizer usage will eventually become prohibitive. Currently, the subject of BNF is of great practical importance because the use of nitrogenous fertilizers has resulted in unacceptable levels of water pollution (increasing concentrations of toxic nitrates in drinking water supplies) and the eutrophication of lakes and rivers (Dixon and Wheeler 1986; Sprent and Sprent 1990). Further, while BNF may be tailored to the needs of the organism, fertilizer is usually applied in a few large doses, up to 50% of which may be leached (Sprent and Sprent 1990). This not only wastes energy and money but also leads to serious pollution problems, particularly in water pollution.

In the case of soils low fertility status, different solutions for improvement of fertility have been considered in several countries, including application of bio-fertilization, nutrients bio-fixation such as atmospheric N_2 fixation by symbiotic nodules between legumes and N-fixing bacteria, or nutrient release from inert state or from minerals bearing nutrients (Schnürer and Rosswall 1987). For instance, inoculation of legume seeds crops with associative N-fixing bacteria led to improvement of plant growth and yield (Rizk and Fatma 2000). Many researchers reported also that using bio-nitrogen-fertilizers in addition to organic fertilizers led to an improvement a vegetative growth and productivity of legumes (Ahmed et al. 2002; El-Bassiony 2002; EL-Etr et al. 2004; Solaiman and Rabbani 2006; Uher et al. 2006; Susheela-Negi et al. 2007). In principle, cultivation of legumes allows symbiotic N_2 fixation, but phosphorus may be a critical constraint for this cultivation. This is due to a substantial need for P in the N_2 fixation process (Tsvetkova and Georgiev 2007). The high requirement for P in legumes is consistent with the involvement of P in the high rates of energy transfer that must take place in the symbiotic nodule. Under P shortage conditions, legumes may lose the distinct advantage of an unlimited source of symbiotic N (Luscher et al. 1998). However, for the common bean (*Phaseolus vulgaris*) some studies revealed a possibility of selecting some cultivars able to support biological N_2 fixation at low level of available P (Pereira and Bliss 1989). These encouraging results led us to conduct a field study with the common bean in the desert soils of Egypt for several reasons. Firstly, common bean plants are considered one of the most important vegetable crops cultivated in Egypt for the local market and it has a great importance for exportation. Secondly, if the genotypic variability of vegetative growth under limited P supply has been verified in some environmental and controlled conditions for common bean, some field effects remain poorly understood. Moreover, bean plants are more sensitive to environmental stresses that may occur in the field (especially under sandy soil conditions) as compared to most vegetable crops. This negatively affects its growth, yield and even the quality of pods. Many investigations have indicated that bean plants are very sensitive to different environmental stresses such as chilling (El-Tohamy et al. 2001; Singer et al. 1996), drought (Millar and Gardner 1972; Halterlein 1983; El-Tohamy et al. 1999) and heat stress (Dale 1964). Common bean has also been considered as species with moderate potential of N_2 fixation, in comparison to other legumes, this is partly due to the susceptibility of bean to nutritional and environmental stresses and to the short period of vegetative fixation (Piha and Munns 1987).

Finally, many investigators reported that mineral fertilizer application is essential for growth, development and yield productivity of bean plants. They indicated that increasing NPK level application improved plant growth, yield and green pod quality of beans (Singer et al. 2000; Saxena et al. 2003; Hafez et al. 2004; Abdel-Mawgoud et al. 2005; Souza et al. 2008). Within this framework, the aim of the present work was to examine in one newly reclaimed desert soil of Egypt and for several common bean RILs, the effectiveness of three levels of mineral phosphorus input on growth, nodulation, yield and the capacity of the plant to fix N_2 by root nodules to mobilize phosphorus in the soil solution for the plant under the drip irrigation system in the winter season of the year of 2008–2009.

Materials and methods

Experimental site

The experiment was conducted at the Agricultural Research Station, National Research Centre, El-Nubaria district, Behera Governorate, Egypt (altitude of 30°30′N and longitude of 30°20′E), in an arid climatic region during the winter season of 2008–2009 in a sandy soil (Typic Torripsamments).

Table 1 Physical and chemical properties of the experimental soil of Nubaria station

Depth	Chemical analysis				Mechanical analysis			Texture
	OM (%)	pH (1:2.5)	EC (dS m^{-1})	$CaCO_3$ (%)	Course sand	Fine sand	Clay + silt	
0–20	0.65	8.7	0.35	7.02	57.76	50.70	2.45	Sandy
20–40	0.40	8.8	0.32	2.34	56.99	39.56	3.72	Sandy
40–60	0.25	9.3	0.44	4.68	36.78	59.40	3.84	Sandy

Depth	Cations (meq L^{-1})				Anions (meq L^{-1})			Available nutrient (mg kg^{-1} soil)		
	Ca^{2+}	Na^{+}	K^{+}	Mg^{2+}	Cl^{-}	SO_4^{2-}	HCO_3^{-}	CO_3^{-}	N	P	K
0–20	0.72	1.65	0.40	0.63	1.90	1.40	0.10	–	38.15	5.12	100
20–40	1.50	0.30	1.60	0.67	2.35	1.71	0.10	–	26.58	7.50	65
40–60	1.49	0.40	2.49	0.76	3.85	1.19	0.10	–	19.30	5.50	45

Some physical and chemical characteristics are given in Table 1 The experimental design was split-split plot design with three replicates. Main plots were occupied with increasing rates of P application in the following manner: phosphorus in the form of phosphoric acid (H_3PO_4); P0 without addition; P1 with 45 kg P_2O_5 ha^{-1} and P_2 with 90 kg P_2O_5 ha^{-1}. The sub-plots consisted of inoculation of rhizobium and sub-sub-plots were assigned with six RILs of common bean plants, three of them being a priori tolerant to phosphorous deficiency (115, 104, 34) and the rest being a priori sensitive (147, 83, 75). The area of each sub-sub-plot was 5 × 2 m, hence the total area of the field experiment was approximately 1,000 m^2. Plants raw spacing was 0.70 m and the distance between plants was 0.25 m.

Soil sampling

Soil samples were collected by auger at the depth of 0–20 cm. These samples were air-dried, crushed, and sieved by 2-mm sieve and preserved for analysis. To assess the soil characteristics in the best possible way, the following methods were used: particle size distribution was carried out using the pipette method and total carbonate for soil as described by (Dewis and Fertias 1970). Total soluble salts were determined by measuring the electrical conductivity in the extraction of saturated soil paste in dS m^{-1} as explained by (Jackson 1967). Amounts of water-soluble cations (Ca^{+2}, Mg^{+2}, Na^{+} and K^{+}) and anions (CO_3^{2-}, HCO_3^{-} and Cl^{-}) were determined in the extraction of saturated soil paste by the methods described by (Hesse P 1971). SO_4^{2-} ions were calculated as the difference between total cations and anions. Soluble Ca^{+2} and Mg^{+2} were determined by titration with standardized versenate solution. Soluble Na^{+} and K^{+} ions were determined using a flame photometer. Soluble CO_3^{2-}, and HCO_3^{-} ions were determined by titration with standardized H_2SO_4 solution. Soluble Cl^{-} ions were determined by titration with standardized silver nitrate solution. Available soil nitrogen was extracted using KCl (2.0 M) and determined by using the macro-Kjeldahl method according to Hesse (1971). Available soil phosphorus was extracted with $NaHCO_3^{-}$ (0.5 M) at pH 8.5 and determined colorimetrically after treating with ammonium molybdate and stannous chloride at a wavelength of 660 nm, according to (Olsen and Sommers 1982). Available soil potassium was determined by extracting soil with ammonium acetate (1.0 M) at pH 7.0 using flame photometer as described by Hesse (1971).

Irrigation setup and cultivation

The drip irrigation lines consisted of drippers spaced 0.50 m apart with a flow capacity of 4 l h^{-1} at 1.5 bar working pressure and the spacing between lateral lines was 0.70 m. The irrigation water source was from ground water. Water samples were collected at every irrigation interval and were subjected to the following analyses: pH, electrical conductivity (EC), and the sodium absorption ratio (SAR) following Richards (1962). Table 2 provides the characteristics of the irrigation water.

Table 2 Chemical characteristics of irrigation water at the Agricultural Research Station of Nubaria

pH	EC (dS m^{-1})	Cations and anions (meq L^{-1})								SAR%
		Cations					Anions			
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_2^{-}	
7.3	0.41	1.0	0.5	2.4	0.2	–	0.1	2.7	1.3	2.8

Six RILs of common bean were cultivated with the spacing of 0.7 m between the ridges and 0.25 m between the plants in a ridge. In the process of fertigation the seedlings were treated with two rates of phosphoric acid (38%) that were injected weekly through irrigation water over the entire growth period of the plant. Moreover, nitrogen and potassium were added in the combined form 20N–0P–5K through the drip irrigation system from the second week until the seventh week of the transplanting stage. Whereas, the form 10N–0P–36K were applied from the eighth week till the end of fertigation program according to the Egyptian Ministry of Agriculture recommendations (N, P_2O_5 and K_2O kg ha^{-1}). The combined fertilizers were produced by Fertilizers Development Center, El-Delta Fertilizers Plant, Egypt.

Parameters measured

The following plant growth parameters were measured: plant height (cm); number of branches; shoot, root and nodule biomass (g $plant^{-1}$); nodule numbers and leaf area (m^2).

N and P contents were determined for shoots, nodules and seeds of the six studied RILs of common beans.

Plant analyses

At harvesting stage, a random sample of nine plants were chosen from each plot and prepared for chemical analysis. These plant samples were dried at 70°C; ground using stainless steel equipments to analyze N and P content of shoot, nodule and seeds. From each sample 0.2 g was digested by the mixture of sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids (1:1) and phosphorus was determined colorimetrically at wavelength 430 nm using a spectrophotometer (Spekol) as described by (Cottenie et al. 1982) while, N was determined according to macro-Kjeldahl method according to Hesse (1971).

Soil analyses

After harvest stage, the soil samples were collected at the depth of 0–20 cm, from each plot. Available phosphorus in the soil extracted by 0.5 M $NaHCO_3$ at pH 8.5. 100 g of rhizospheric and bulk soils were preserved in sealed plastic bags and stored in a cold room at 4C° till analyses. In each plot, Olsen-P of rhizosphere and bulk soil was measured according to Malachite green Pi method (Olsen and Sommers 1982), 0.5 g of dried soil extracted by 0.5 M $NaHCO_3$ at pH 8.5. Rhizosphere pH was measured with a Metrohm-744 pH meter with a combined glass electrode after extractions in deionized water for 5 min and measured in the suspension of the soil extract of soil: water ratio of 1:2.5 (AFNOR 1999). Net proton release in the rhizosphere by roots was calculated according to the pH change relative to the no-plant control soil and taking account of the soil pH buffer curve.

Statistical analysis

Standard analysis of variance technique was used to analyze the means of treatments following (Gomez and Gomez 1984). The means were differentiated according to Duncan's multiple range tests and represented with letters in the tables (Duncan 1955).

Results and discussion

Growth and nodulation

As illustrated by Table 3, P supply, RILs and interaction between P supply and RILs affected highly significantly the studied parameters of plant growth except the number of branches which remained between 4 and 5. The highest mean value of plant height 49.58 cm was observed in RILs

Table 3 Plant growth of common bean plants affected by genotypes and P application rates of Nubaria station

Treatments	Plant height (cm)	No. of branches	Dry weight (g $plant^{-1}$)				LA (m^2)
			Shoot	Root	Nodule	Nodule number	
Genotypes							
34	49.58a[a]	5.17	15.1ab	3.62a	0.60ab	30.00b	0.439d
147	49.58a	4.61	9.7b	2.98a	0.73a	21.33b	0.573b
83	33.44b	4.11	14.4b	3.34a	0.60ab	51.22a	0.565c
75	31.36b	4.83	21.4a	3.47a	0.65ab	29.67b	0.607a
104	41.06ab	5.00	8.8b	3.56a	0.52b	21.44b	0.585b
115	32.29b	5.06	12.7b	2.94a	0.51b	23.89b	0.502d
P application rates							
P0	35.72b[a]	4.97	12.2a	2.82b	0.54b	20.33b	0.452c
P1	37.04b	4.78	14.1a	3.17b	0.62ab	31.11a	0.583b
P2	45.83a	4.64	14.7a	3.97a	0.64a	37.33a	0.584a

[a] Means within a column followed by the same letters are not significantly different according to the Duncan multiple range test ($P < 0.05$)

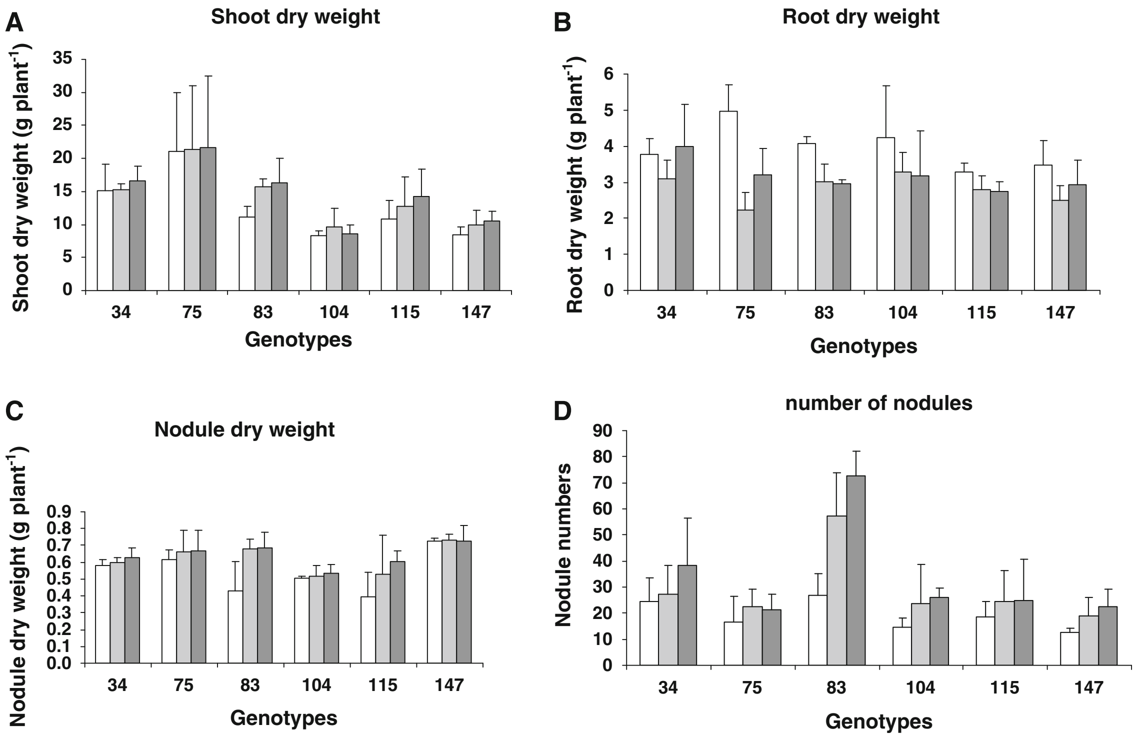

Fig. 1 Effect of P nutrition on **a** shoot, **b** root biomass, **c** nodule biomass and **d** nodule number in six common bean RILs in Nubaria. Data are means and SD of 9 harvested plants. P0 versus P1 and P2

34 and 147. RIL 75 had a significant higher leaf area (0.607 m^2) as compared to other RILs. Table 3 illustrated a positive interaction between P supply and RILs, whereas the strong impact of P supply was observed under P level of 90 kg P_2O_5 ha^{-1}.

Furthermore, the P supply, RILs and the interaction between P supply and RILs affected significantly the shoot biomass. Figure 1a shows an increase in shoot biomass with the higher level of P by more than 30% for RIL 83 and about 15% for RILs 115 and 147 as compared to the control (without P). By contrast Fig. 1b shows a decrease in the root biomass with the higher level of P by more than 30% for RILs 83, 104, 115 and 147 and more than 60% for RIL 75 as compared to the control. The nodule biomass increased significantly by interaction between increasing P supply and efficient RILs. Figure 1c shows an increase in nodule biomass with the higher level of P by more than 40% for RILs 83 and 115 and about 20% for RILs 75 and 104 as compared to the control. The P supply, RILs and interaction between P supply and RILs affected significantly the nodule biomass and nodule numbers. Figure 1d shows a significant increase with the higher level of P in nodule numbers: nodule numbers were more than twofold higher as compared to the control for RIL 83, more than 50% for RIL 34 and about 30% for RILs 104, 115 and 147 compared to control.

Figure 2 shows a positive significant correlation between nodules biomass and nodule numbers for common bean RILs. A highly significant relation can be observed in RIL 104 ($r = 0.85$, $n = 9$, $p < 0.05$), followed by RIL 34 ($r = 0.82$, $n = 9$) and RIL 83 ($r = 0.79$, $n = 9$).

Correlation between nodulation and plant N content and growth

Figure 3 shows that there was a positive significant correlation between nodule biomass and N content of the plant ($r = 0.84$, $n = 6$). This is explained by the effect of rhizobial symbiosis in the nodules for N_2 fixation. Moreover, Tables 3 and 4 reveal the effect of RILs and P supply on nodule biomass, shoot biomass and N content of the plant with the highest values for N content recorded in RIL 75 followed by RILs 104 and 83. Finally, on multiplying the shoot dry weight (Table 3) by the nitrogen content of the shoots (Table 4), it has also been observed the RIl 75 accumulates the highest amount of nitrogen per plant (0.69 g $plant^{-1}$) whereas treatment P2 provides more N in shoots than the other levels (around 0.43 g in shoot biomass per plant for P2, followed by 0.39 g in shoot biomass per plant for P_1 and 0.37 g for P_0).

Plant growth depended upon nodule SNF since N_2 was the source of N for plant nutrition. Therefore, the efficiency

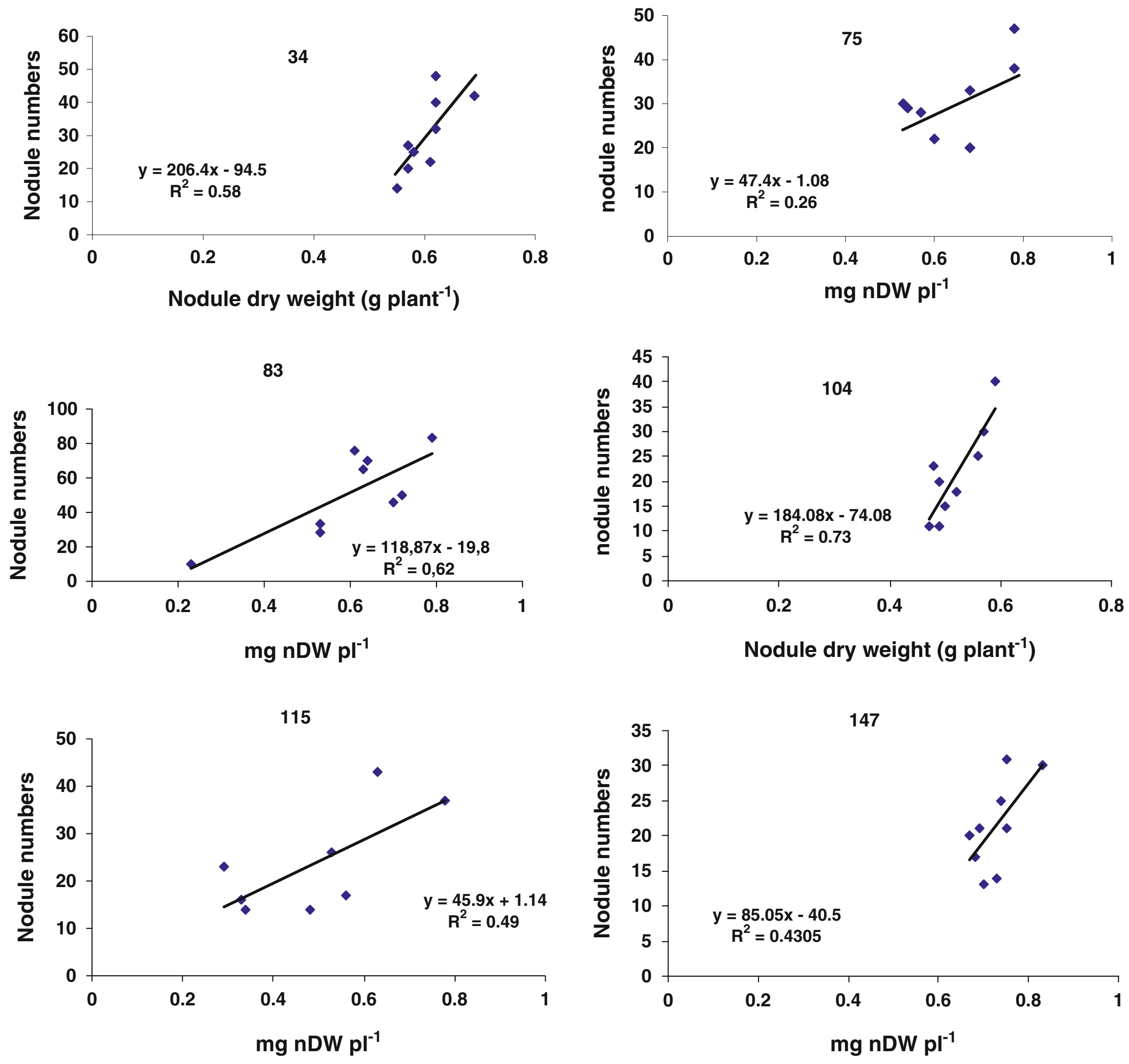

Fig. 2 Correlation between nodule biomass (g plant^{-1}) and nodule numbers in six common bean RILs. Data are means and SD of 9 replicates harvested plants

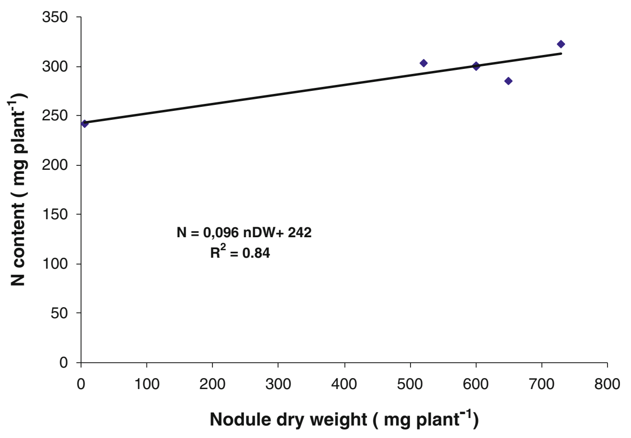

Fig. 3 Efficiency in use of rhizobial symbiosis of six common bean RILs under three phosphorus levels. Data are means of 9 harvested plants for each RIL

in utilization of the rhizobial symbiosis (EURS) was assessed by the regression slope of the relation of shoot growth as a function of nodule biomass (Zaman-Allah et al. 2007; Bargaz et al. 2011). The data in Fig. 4 show that considering all the tested RILs, shoot and nodule DW were positively correlated under phosphorus supply levels with EURS being the highest for RIL 75 than other RILs, with a highest value of $r = 0.89$ ($p < 0.05$) followed by RIL 34 with value of $r = 0.81$ ($p < 0.05$) and 104 with value of $r = 0.78$ ($p < 0.05$).

Furthermore, the analysis of variance (ANOVA), with results provided in Table 3, confirmed the significant effect of P supply and RILs on plant growth, and nodulation. RIL 75 shows the highest shoot biomass (21.4 g plant$^{-1)}$, compared to other RILs followed by RIL 34 (15.1 g plant^{-1}), and (14.4 g plant^{-1}), for RIL 83. Furthermore, the effect on nodulation was the most significant for RIL 147 (0.73 g

Table 4 Means of N and P content (%) of common bean plants and Olsen-P and pH of soil as affected by genotypes and P application rates of Nubaria station

Treatments	P (%)			N (%)		Soil Olsen-P (mg kg^{-1})	pH
	Shoot	Nodule	Seeds	Shoot	Seeds		
Genotypes							
34	0.288ab	0.396b	0.680a	3.00b	3.31b[a]	14.1c	7.82ab
147	0.247b	0.418b	0.422ab	2.85c	3.33b	40.6a	7.87a
83	0.338a	0.381b	0.336b	3.01b	3.31b	33.7ab	7.64c
75	0.253b	0.480a	0.418ab	3.22a	3.18c	41.9a	7.70bc
104	0.268b	0.360b	0.388ab	3.03b	4.06a	30.7b	7.88a
115	0.267b	0.415b	0.350b	2.42d	3.32b	38.3ab	7.94a
P application rates							
P0	0.240c	0.390b	0.375c	3.04a	3.46b[a]	28.4b	7.85a
P1	0.278b	0.405ab	0.400b	2.80c	3.27c	29.9b	7.87a
P2	0.313a	0.430a	0.521a	2.92b	3.53a	41.4a	7.71b

[a] Means within a column followed by the same letters are not significantly different according to the Duncan multiple range test ($P < 0.05$)

plant^{-1}) followed by RIL 75 (0.65 g plant^{-1}) and RIL 83 (0.60 g plant^{-1}). Also positive significant relation between nodule numbers and P supply has been observed where RIL 83 had the most nodule numbers (51 nods. plant^{-1}).

Phosphorus concentration in shoot and nodule, and phosphorus use efficiency

P supply, RILs and interaction between them affected significantly the P concentration in shoots and nodules. Figure 5a shows that the shoot P content was more than 60% for RIL 83 as compared to the control, and more than 30% for RILs 34, 104, 115 compared to control. Furthermore, Table 4 reveals that the highest P content was observed with RIL 83 (3.38 mg g^{-1}) with high input of P (90 kg P_2O_5 ha^{-1}). Furthermore, as shown by Fig. 5b, the P concentration in nodules was more than 40% for RIL 115 as compared to the control and about 20% for RILs 34 and 83. Moreover, Table 4 reveals that the most P content of nodule was observed in RIL 75 (4.8 mg g^{-1}) under high input of P (90 kg P_2O_5 ha^{-1}).

The interaction between P supply and efficient RILs indicated highly significant effect of P input on P content in shoots and nodules. Figure 5c illustrates that the variation between the six common bean RILs in the use of phosphorus is in the biological nitrogen fixation. The highest value was observed in RIL 75 followed by RIL 34; moreover, the highest input of P corresponds to a higher increase in the mean values of shoots, nodules and seeds P contents in all investigated RILs.

Rhizospheric soil acidity

As shown by Table 1 the soil pH measured before cultivation was 8.7. Table 4 indicates that there was a significant reduction in pH after cultivation for all RILs of common beans: the pH was reduced until 7.64 with RIL 83 followed by 7.70 with RIL 75 and 7.82 with RIL 34 during the growing season (this can be connected to an efflux of protons from the roots). Also, the available P increased in concordance with an increase in H^+ efflux and consequent decrease in pH under the highest P input (90 kg ha^{-1} P_2O_5), with an increase in soil Olsen-P and become available P for plants.

Nodule and shoot biomass as a function of Olsen-P

Figure 6 shows that nodule biomass of different RILs as a function of Olsen-P concentration with nine replicates of harvested plants for each RIL. It indicates that all RILs have a positive correlation with soil Olsen-P with the highest $r = 0.84$ for RIL 147 followed by RIL 75 where $r = 0.79$ and RIL 83 where $r = 0.78$ followed by RIL 34 where $r = 0.78$ followed by RIL 115 where $r = 0.73$ and the lowest value of $r = 0.66$ for RIL 104. Moreover, Table 3 reveals that the highest Olsen-P was observed in RIL 75 (41.9 mg P of kg^{-1} soil) followed by RIL 147 (40.6 mg P kg^{-}1 soil).

For RILs 115 and 83, the nodulation increased with Olsen-P till a maximal value of about 90 mg of nodules per plant, corresponding to the critical P value of 30 and 40 mg of Olsen-P by kg of soil. For each increase of 1 mg P kg^{-1} of soil, the nodulation of RILs 115 and 83 increased by 17 and 19 mg of nodule biomass per plant, respectively. For the RILs 34, 75, 104, 147, the relationship was linear with nodulation, increasing till the maximal value corresponding to the critical P ranging between 30 and 40 mg kg^{-1}. The maximal values of nodulation given by these RILs were 70, 80, 60 and 80 mg plant^{-1}, respectively. For each increase of 1 mg P kg^{-1} of soil the nodulation of these RILs increased by 4, 4, 8 and 3 mg nodule biomass per plant, respectively.

Figure 7 shows the shoot biomass of the different RILs as a function of Olsen-P of the experiment, with nine

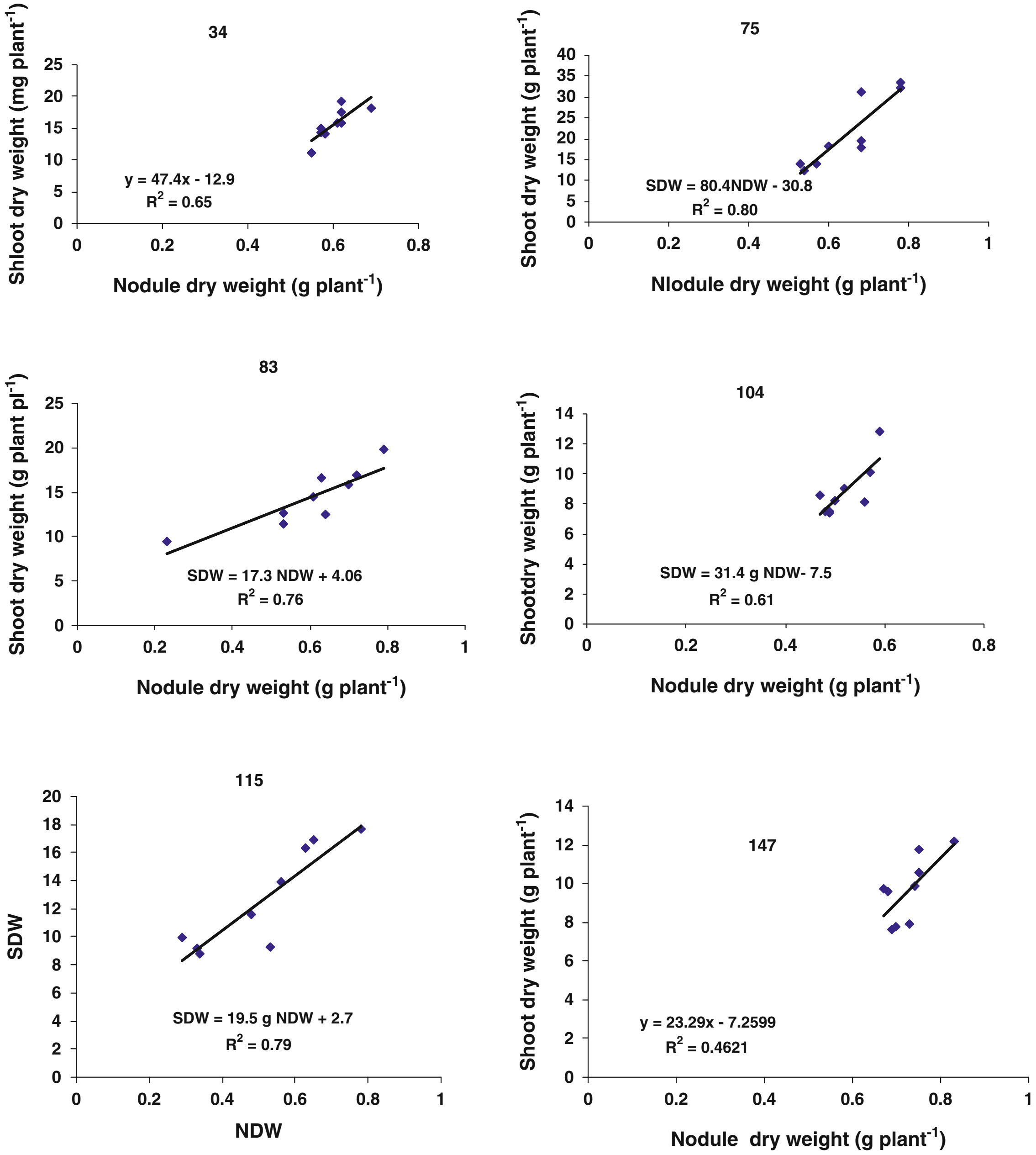

Fig. 4 Effect of P nutrition on use efficiency of symbiotic nitrogen fixation (EUSR) (the regression parameter of shoot as a function of nodule) of the RILs in Nubaria. Data are means SD of 9 replicates harvested plants

replicates of harvested plants for each RIL. The shoot biomass increased significantly with Olsen-P till maximal value of $r = 0.83$ for RIL 83 followed by RIL 34 where ($r = 0.82$) and RIL 104 where ($r = 0.81$). The weakest correlations were observed in RIL 75 with $r = 0.49$ and in RIL 147 with $r = 0.63$. This contrasts with the correlation between nodule biomass and concentration of Olsen-P, as the maximal values for this correlation were observed in RILs 75 and 147. The maximal shoot biomass was about 21 g plant^{-1} for RIL 75 with the highest Olsen-P of 41.9 mg P kg^{-1} soil.

Furthermore, shoot biomass increased significantly with Olsen-P till the maximal value corresponding to the critical value of 18 and 23 mg Olsen-P kg^{-1} of soil for RILs 83 and 115. For each increase of 1 mg P kg^{-1} of soil the shoot biomass of RILs 83 and 115 increased by 0.4 and 0.6 g of shoot biomass per plant, respectively. On the other hand for RILs 34, 75, 104, and 147 the maximal values corresponding to the critical P were 30, 80, 35 and 55 mg Olsen-P kg^{-1} of soil, respectively. By contrast the critical values were 5, 22, 23 and 25 mg P kg^{-1} of soil, respectively. The maximal values of shoot biomass were 20 g for RILs 34

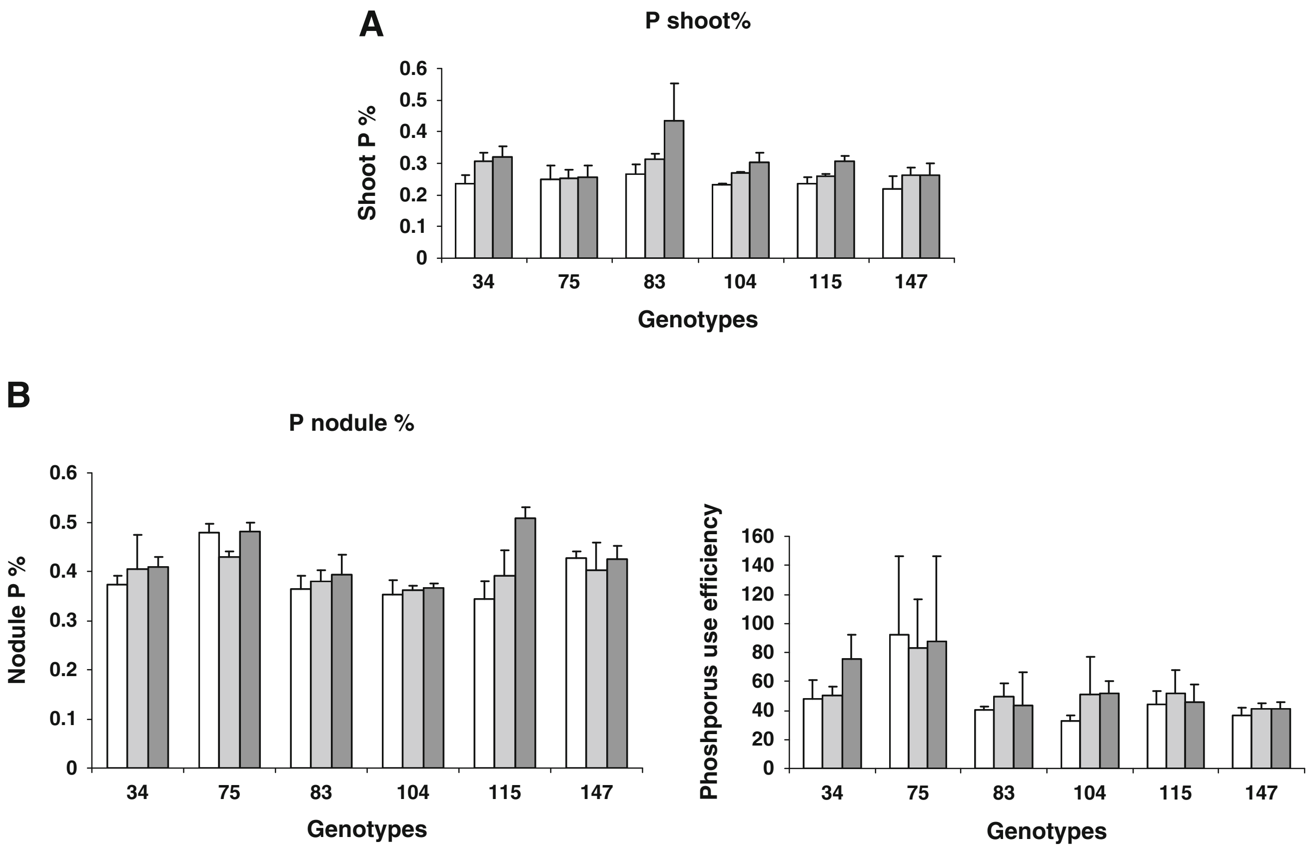

Fig. 5 Effect of P nutrition on **a** shoot and **b** nodules and phosphorus use efficiency of six bean RILs in Nubaria. Data are means SD of 9 replicates of harvested plants

and 104, 30 g for RIL 75 and 12 g for RIL 147, respectively. Nevertheless, each increase of 1 mg P kg^{-1} of soil for RILs 34, 75, 104, 147 was associated with an increase in shoot biomass of 0.2 to 0.25, 1.04 and 0.16 g $plant^{-1}$, respectively.

Discussion

In this study, the difference between the contrasting RILs in PUE for symbiotic nitrogen fixation (SNF) was investigated. Furthermore, the quality that bio-availability of phosphorus is affected by nodulation growth and N_2-dependent growth of the legume, was verified. RILs tolerant to a deficiency of P versus strain sensitive to a deficiency of P were also investigated. The results show significant relationship between plant growth and the formation of the root nodules, and also significant differences of these relations between RILs.

These results can be explained in term of differences between RILs in P requirement for N_2 fixation and difference in PUE. They agree with previous results obtained for the common bean (Vadez and Drevon 2001) and for the soybean (Gunawardena et al. 1996) and *Acacia mangium* (Vadez et al. 1997). The PUE concept, initially defined by Siddiqi and Glass (1981) and discussed by Gourley et al. (1993), has gained scientific interest (Israel and Rufty 1988). It may also have economic significance for the management of chemical fertilizers in highly productive environments or in marginal land where the cost of fertilizer limits its use. Vadez et al. (1999) found that genotypic differences for N_2 fixation under P deficiency were most likely due to differences in phosphorus use efficiency.

In this study, existence of the linear correlation between nodulation and shoot biomass was the highlight, indicating that nodules were sufficiently efficient. Nitrogen nutrition of legumes, which relies on atmospheric N_2 and soil mineral N, remains a major limiting factor of growth (Voisin et al. 2007). A decade ago, breeders tried to increase N uptake through hyper nodulation (Sagan and Duc 1996; Voisin et al. 2007). The reduction of the atmospheric nitrogen by nitrogenase requires large energy expenditure with a half to be provided by ATP. This molecule is indeed necessary for the transfer between components of the nitrogenase complex, of electrons that will be eventually allocated to a nitrogen atom for ammonium synthesis.

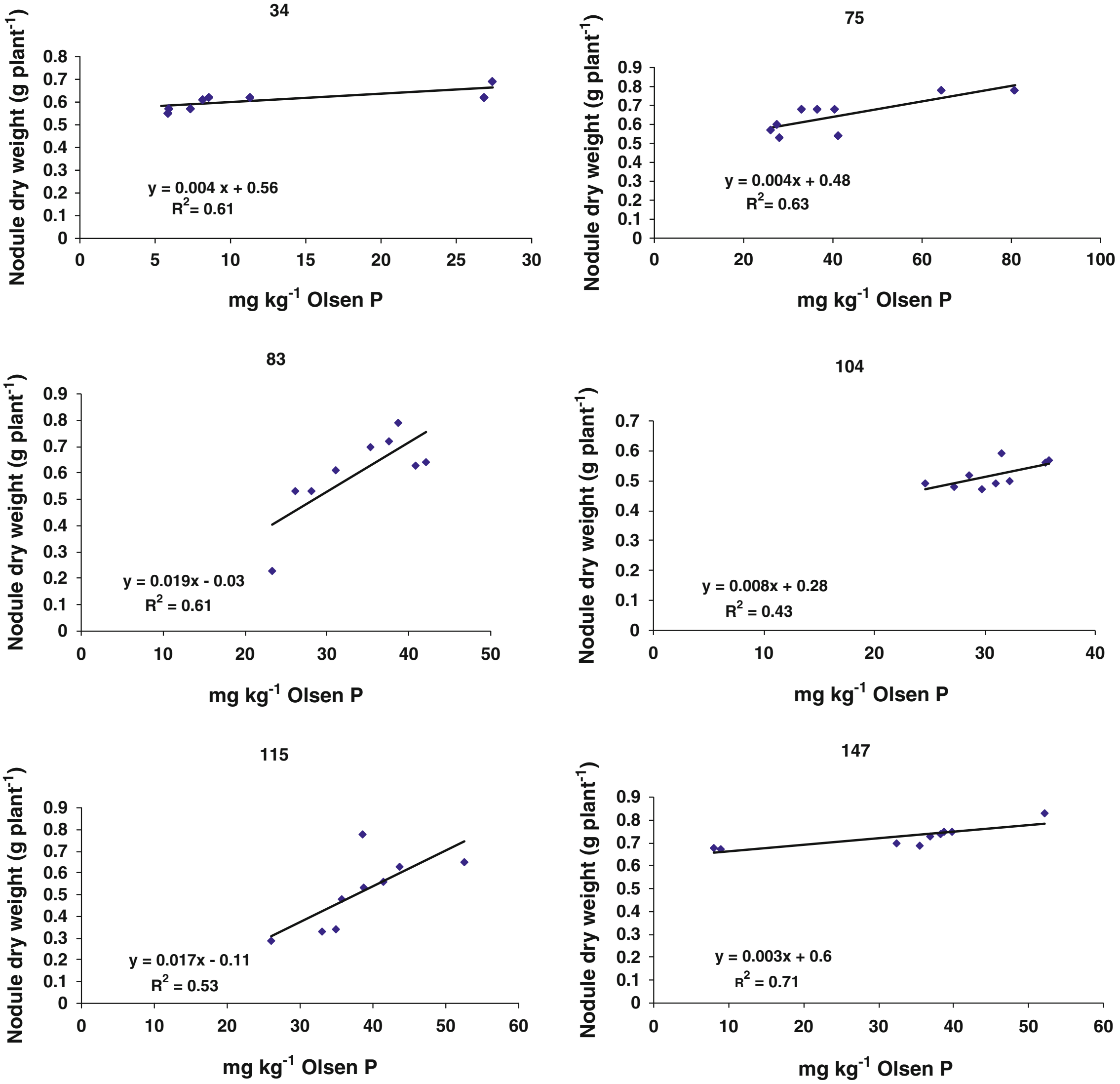

Fig. 6 Nodule biomass versus concentration of Olsen-P in soil, of six common bean RILs grown in Nubaria station. Data are means and SD of 9 replicates of harvested plants

In the rhizobial legume symbiosis, the synthesis of this ATP depends on phosphorus (Ribet and Drevon 1996).

Legumes depending on symbiotic N_2 fixation have a higher internal P requirement for optimum nitrogen accumulation than plants dependent on combined N (Ribet and Drevon 1996). Several authors have showed that P deficiency can impair nodulation and N_2 fixation (Tang et al. 2001; Valverde et al. 2002). In plants relying on N_2 fixation, P stimulated nodulation and N_2 fixation more than it stimulated plant growth (Israel 1987; Leidi and Rodriguez-Navarro 2000; Hellsten and Huss-Danell 2001; Gentili and Huss-Danell 2002, 2003; Valverde et al. 2002). Alternatively, stimulation of nodulation by P might be ascribed to a general growth effect in legumes (Robson et al. 1981; Jakobsen 1985) and in an actinorhizobial symbiosis (Reddell et al. 1997). Thus, although RIL 147 was the most nodulated RILs in this study, its overall lower efficiency in use of the rhizobial symbiosis suggested that a lack of nodulation of the RILs could be compensated by an increase in the nodule efficiency to support plant growth. In most studies the positive response of plant growth and symbiotic N_2-fixation to increased P supply in legumes is thought to be because symbiotic N_2-fixation is an energy-demanding process with a high ATP requirement of 16 ATP for reduction of one molecule of N_2 into $2NH_3$ (Israel 1987; Sanginga et al. 1989; Burris 2000).

The influence of P availability on N_2-fixation was emphasized by the fact that during P stress in white lupin, 80% of new nodule growth was preferentially located near cluster roots (Vance et al. 2003) where P uptake is higher than non-cluster root parts of the root. Also P-deficient legumes may modulate N_2-fixation through low sink demand for N (Hartwig 1998; Almeida et al. 2000) as a

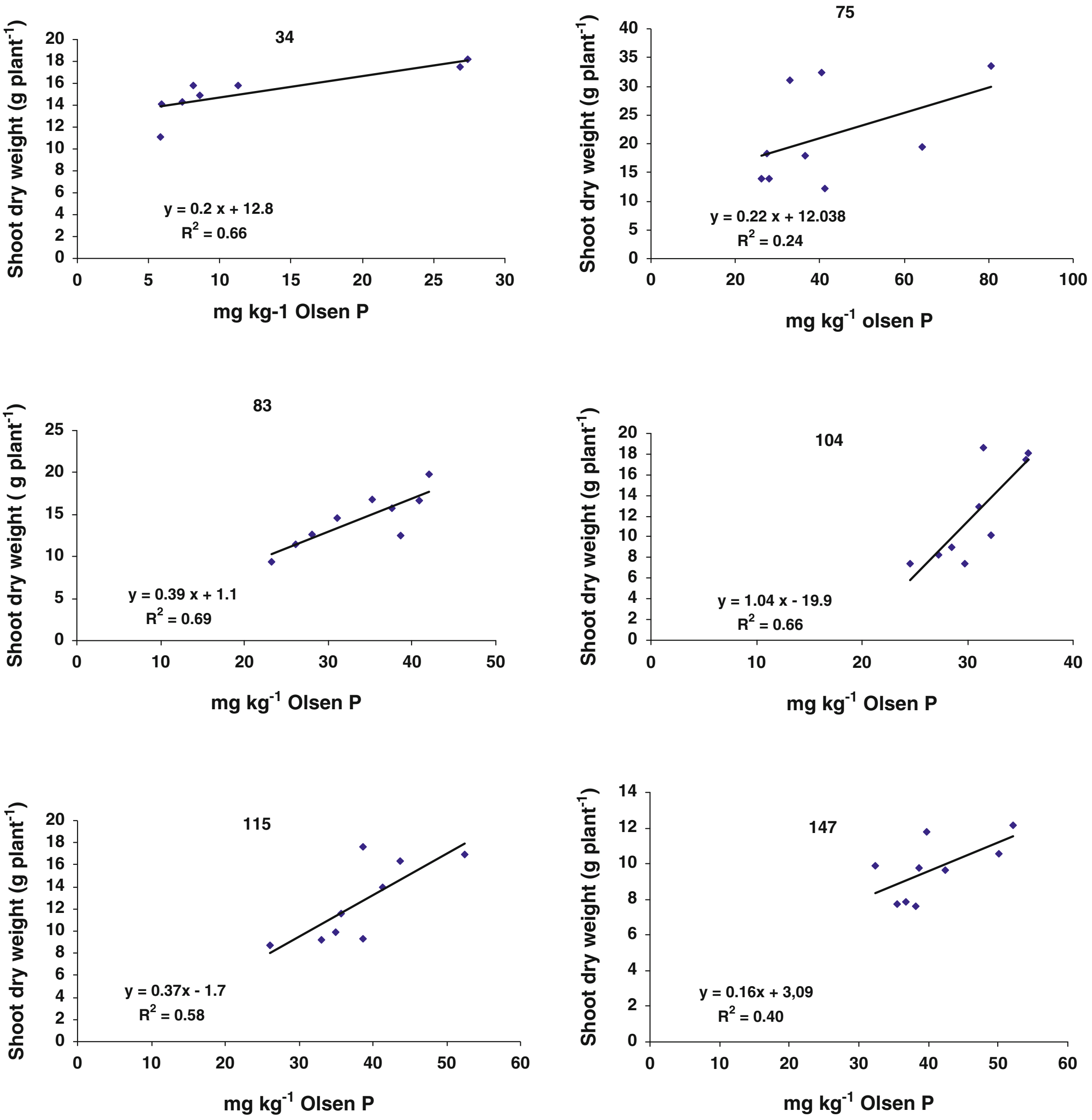

Fig. 7 Shoot biomass versus concentration of Olsen-P in soil, of six common bean RILs grown in Nubaria station. Data are means and SD of 9 replicates of harvested plants

response to decreased shoot growth through lowered leaf expansion and lowered specific leaf area (Jensen and Hauggaard-Nielsen 2003). Moreover, the relation which linked nodule biomass and shoot biomass of six RILs of common bean to Olsen-P concentration in soils presented systematically a best fit with a linear regression, by increasing from 0 to 45 mg of P kg^{-1} soil, and by being quite constant or decreasing beyond this value of P. P would limit nodulation, whereas above 45 mg of P kg^{-1} soil, the P availability would be higher than the nodule requirement for most RILs, and eventually become toxic.

Thus P toxicity is apparent in low-P adapted plants when grown experimentally at relatively higher P supply, although the extra P could be stored in the vacuole (Schachtman et al. 1998; Shane et al. 2004). However, this study shows that legumes would scarcely encounter such high concentrations of Olsen-P toxicity in Mediterranean fields. The contrasting RILs 34, 104 and 147 would be less demanding for P than the other RILs under study, since its relatively high nodulation was not affected by Olsen-P variation between 20 and 50 mg of P kg^{-1} soil among sites. RILs 75, 83 and 115 would be the most P demanding since their nodulation was still responding to an increase of P above 50 mg of P kg^{-1} soil. Overall, RIL 75 showed highly significant values for vegetative growth, N and P content of nodule and seeds and increased availability of P, as well as the largest leaf area content. It is followed by RILs 147 and 83 then other three RILs of common beans.

Conclusion

The results showed notably that shoot biomass produced by common bean was related to the nodulation rate of roots. This demonstrates the efficiency of rhizobial symbiosis in six RILs. Moreover, specific analyses showed that nodulation rate, and thus shoot biomass; depend on available P. These RILs have the ability to reduce the amount of nitrogen fertilizer via fixing N_2 from the atmosphere by their root nodules. This is interesting because N fertilizer is expensive and this type of soil has difficulty in providing nutrients, Therefore, these RILs appears as suitable to this newly reclaimed sandy soils. Some RILs are interesting to study (e.g., RILs 75, 83). These results allow us to consider conducting future experiments with these RILs.

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