



Original Research Article

Effect of rhizobial inoculation on nodulation and plant growth of faba bean in Tunisian farmer's field test

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Osmotic constraints, nutrient deficiencies, especially phosphorus, and the lack of efficient strains of rhizobia in Mediterranean soils are major factors limiting symbiotic nitrogen fixation and yield in faba bean (*Vicia faba* L.var. minor). In order to improve faba bean plant growth and nitrogen uptake in a multi-local field test, experiments were carried out in 2012/2013 in twelve farmer's fields in different environments with the commercial cultivar Bachar of faba bean and was inoculated with a local rhizobial strain Mat.11. Nodulation, plant growth, efficiency in use of the rhizobial symbiosis, nitrogen accumulation and the effect of soil phosphorus (P) availability on plant growth analysis were measured in inoculated and non inoculated treatments. The results indicate a large spatial variation in nodulation and plant growth with the rhizobial strain inoculated under field conditions. Moreover, we noticed a correlation ($r^2 = 0.45$) between the rhizosphere P availability and efficiency in use of the rhizobial strain. In many sites, the inoculation with Mat.11 increased significantly not only nodule number and biomass but also the total plant nitrogen uptake comparing to without inoculation treatment. It is concluded that faba bean inoculation improve significantly nodulation and plant growth. Further investigations should address the variation SNF among local genotypes and the symbiosis mechanisms to stabilize the legume production in soils with low fertilizer input.

Key words: Correlation, nitrogen, nodulation, phosphorus, Tunisia.

INTRODUCTION

In Tunisia, food legumes are a basic component in traditional farming systems. They intervene directly in the ration food or indirectly in feeding livestock. Legumes occupy less than 5% of the areas of cereals in Tunisia and covers a surface area of about 56,600 ha Ministry of agriculture, (2011). Legumes play a major environmental role in cropping systems, principally as a source of symbiotic fixation of atmospheric nitrogen that may save nitrogen fertilizer and also in weed and pest management in crop rotations Graham (2008). Environmental constraints such as high soil acidity and low soil nitrogen and P levels considerably limit bean production, in

particular in the Mediterranean and tropical zones (Graham and Vance, 2003). Several nutrients are essential for plant legume growth and rhizobia specific roles in symbiotic N₂ fixation (SNF) since these bacteria significantly affect nodulation and then N₂ fixation process (O'Hara et al., 1988; Lazali and Drevon, 2014). However, the SNF seems to be highly decreased under constraint conditions and more particularly under P-deficiency (Lazali et al., 2016), as major yield-limiting nutrients in many regions of the world (Pereira and Bliss, 1989; Camila and Lazara, 2004). Thus, low soil-P availability is a primary constraint to agricultural productivity in many low-input systems. The P deficiency

affects particularly the rhizobial symbiosis. Nodules are a strong sink for P with three times higher P concentrations than other organs (Vadez et al., 1996). Consequently, legumes have higher P requirements than non symbiotic plants Israel (1987). The P deficiency might be mitigated by the application of mineral fertilizers that provide soluble Pi for legumes. However, this practice is inherently inefficient because most of the P applied to soils is converted into unavailable forms of P that cannot be easily acquired by plant roots (Abel et al., 2002). Consequently, combining adequate nutrient fertilization, especially P, and efficient native rhizobia may have a synergistic effect in order to improve SNF and the subsequent production of faba bean. Thus, the aim of this work was to assess, under field conditions, the response of faba bean to local rhizobial inoculation without additional nitrogen in terms of nodulation, growth parameters N₂ fixation and its interaction with soil P availability.

MATERIALS AND METHODS

Multi located field trials

The study was conducted during 2012-2013 in 12 farmer's field sites with mean distances of 20 km between each site at Mateur, eastern north of Tunisia (Latitude: 37°02'N, Longitude: 9°39'E and altitude 36 m. Two treatments, each on an elementary plot of 100 m², were compared in twelve field sites: non inoculated (control) and inoculated with Mat strain (Mat.11) containing ten rows distant of 50 cm at a sowing density of 30 seeds per m and cultivated with Bachar cultivar selected by INRAT. In order to prepare fresh inoculum containing the same number of bacterial population for all rhizobia under study, a colony of Mateur rhizobia was transferred into Erlenmeyer containing 100 ml of Yeast extract mannitol medium (Vincent, 1970). Inoculated flasks were incubated at 27°C on a rotary shaker (150 rpm) for 72 hours. All bacterial suspensions were adjusted to about 10⁸ CFU ml⁻¹. This suspension was used to inoculate the emerging seedlings using a watering can. Plants were irrigated by rainfall regime.

Plant growth evaluation

Nodulation and growth of the plants were measured at the flowering stage (60 days) when the first pod was 2 cm long for 50% of the plant population. Sampling was performed by excavating 20 cm in depth and around the root system of 20 plants per row and per block. The plants were separated into shoots, nodules and roots. Roots and nodules were carefully separated from surrounding soil. Shoots and nodules were dried at 60°C for 3 days before determining their dry weights (DW). The efficiency in use of the rhizobial symbiosis (EURS) was estimated as the slope of the linear regression model of shoot biomass as a function

of nodule biomass (Lazali and Drevon, 2014).

For N determination, the sample was digested in hot concentrated H₂SO₄, as described by Kjeldahl. Soil P availability was determined after extraction in NaHCO₃ (Olsen et al., 1954)

Statistical analysis

All data collected were subjected to one way analysis of variance (ANOVA) using the SPSS software (SPSS Inc., Chicago, USA). The mean differences were compared by the Duncan's test at 5% probability. The relationship between different parameters was tested by regression analysis with SPSS software.

RESULTS

Effect of rhizobial inoculation on nodulation

Data analysis showed that without inoculation, the nodule biomass varied significantly among field sites from 0.50±0.05 g nodule DWplant⁻¹ in A9 to 0.10±0.01 g nodule DWplant⁻¹ in A1 (Table 1). Faba bean inoculation improved significantly the nodulation where the nodule biomass varied from 0.60±0.04 g nodule DWplant⁻¹ in A1 to 0.100±0.02 g nodule DWplant⁻¹ in A10. The inoculation significantly increased the nodule biomass in the sites A1, A6, A7 and A8. Overall, the lowest nodule biomass was observed in the sites without inoculation, and the inoculation increased the mean nodule biomass by 42%. Nevertheless, the inoculation decreased significantly the nodulation in the sites A5 and A9 where was observed the highest nodule biomass without inoculation. Without inoculation, the nodule number varied from 90±0.04 nodules plant⁻¹ in A2 to 40±0.01 nodules plant⁻¹ in A7. The inoculation increased significantly the nodule number in A1 up to 144±0.10 nodules plant⁻¹ whereas; it decreased significantly in the sites A2 and A11. However, the lowest nodule number was observed in the sites without inoculation.

In order to assess the individual nodule biomass, the mean values of number and biomass of nodule per site were plotted in Table 1. Significant regressions of nodule biomass as a function of nodule number were found for both treatments. Without inoculation, three significantly different regression-slopes, i.e. the mean individual nodule biomass, were found: 8.00±0.01 mg nodule plant⁻¹ in A1, A6, A8 and A7; 5.50±0.01 mg nodule plant⁻¹ in A4, A3, A11, A9, and A5; 5.10±0.02 mg nodule plant⁻¹ in A12, A2 and A10. With inoculation a significant regression of nodule biomass as a function of nodule number was found in A1, A6, A8, A11, A5 and A7 with mean individual nodule biomass of 14.00±0.02 mg nodule plant⁻¹. However, a negative effect of the inoculation on the individual nodule biomass was observed in the sites A10, A12, A4, A2, and A5.

Table 1. Nodule dry weight and its correlation with nodule number for cultivated faba bean in field sites. Data are means and SD of 20 replicates harvested at flowering stage without or with inoculation

site	nod Nb c	Nod Nb i	NDW c (g)	NDW i (g)
A9	64,5±2,12	50,3±8,18	0,505±0,26	0,291±0,16
A5	59±19,79	43,8±12,44	0,45±0,11	0,187±0,11
A8	56±7,07	72,1±24,11	0,33±0,01	0,48±0,16
A12	85,5±29,59	66,6±22,97	0,235±0,07	0,253±0,13
A4	60±2,82	56,6±31,85	0,18±0,11	0,251±0,14
A6	59±15,55	61±21,85	0,175±0,03	0,495±0,17
A2	92±34,64	43,3±11,98	0,165±0,4	0,254±0,15
A10	66,5±2,82	55,2±11,98	0,135±0,17	0,131±0,1
A7	37,5±14,84	56,9±21,02	0,125±0,13	0,33±0,06
A11	62±23,32	42,5±18,12	0,095±0,01	0,238±0,19
A3	46±1,41	54,3±16,69	0,075±0,03	0,17±0,15
A1	68,5±27,47	144,3±26,85	0,065±0,04	0,598±0,11

Correlation factor			
without inoculation		with inoculation	
A5		A1	
A7	0,99	A2	
A8		A4	
A9		A5	
A3		A6	0,91
A4		A7	
A6	0,66	A8	
A10		A9	
A11		A11	
A1		A3	
A2	0,94	A10	0,67
A12		A12	

Table 2. Shoot dry weight and root dry weight of faba bean in field sites. Data are means and SD of 20 replicates harvested at flowering stage without or with inoculation

site	SDWc (g)	SDWi (g)	RDWc (g)	RDWi (g)
A9	3,8± 0,65	9± 3,35	1±0,65	1,4±0,35
A5	13,6± 2,75	13,8±3,57	0,9±0,34	2,6±0,47
A8	10,9±5,28	19,5±3,70	0,8±0,61	1,5±0,56
A12	5,5±1,17	4,7±1,35	0,9±0,44	1,2±0,34
A4	4,8b±3,52	6,1±3,12	1,4±0,11	1±0,15
A6	3,8±0,03	7,4±0,26	0,9±0,38	1,4±0,51
A2	9,6±2,18	10,4±2,85	1,8±0,05	1,4±0,47
A10	3,2±2,04	2,9±1,57	0,6±0,06	0,6±0,22
A7	4,1±0,04	4,7±0,51	2,1±0,04	1,1±0,51
A11	2,8±0,12	2,9±1,02	0,2±0,05	0,67±0,42
A3	6,1±0,01	13,2±2,43	1,4±0,06	1,9±0,07
A1	2,7±0,29	3,4±2,20	1,6±0,56	1,6±0,47

Effect of rhizobial inoculation on plant growth

Shoot biomass varied from 4.00±0.41 g shoot DW plant⁻¹ in A1 to 13.60±1.02 g shoot DW plant⁻¹ in A5 and from 3.00±0.40 g shoot DW plant⁻¹ in A10 to 19.50±0.50 g shoot DW plant⁻¹ in A8, without and with inoculation, respectively (Table 2). The inoculation increased the shoot biomass from 5% in the site A7, 92% in the site A6, 77% in

the site A8, and 100% in the sites A3 and A9. Overall, the lowest shoot biomass was observed in A1, A11 and A3 in control plot.

Root biomass varied from 2.30±0.21 g root DW plant⁻¹ in A3 without inoculation to 0.50±0.31 g root DW plant⁻¹ in A11, and with inoculation, from 4.30±0.30 g root DW plant⁻¹ in A3 to 1.00±0.14 g root DW plant⁻¹ in A10. The inoculation increased root biomass from 1% in the site A1,

Table 3. Efficiency in use of rhizobial symbiosis without or with inoculation. The equations describe the regression function of 10 replicates harvested at flowering stage.

site	NDW c (g)	SDW c (g)	NDW i (g)	SDWi (g)	EUSR (gNDW SDW)	correlation factor (r ²)
A1	0,06	3,41	0,47	1,12	4,30	0,66
	0,08	3,42	0,20	1,65		
	0,04	3,35	0,15	1,99		
	0,06	4,26	0,19	1,50		
	0,05	4,16	0,63	6,03		
	0,10	4,50	0,58	2,06		
	0,15	3,43	0,58	2,77		
	0,06	3,50	0,68	5,10		
	0,06	4,63	1,00	6,19		
	0,09	5,13	1,50	6,57		
A11	0,25	4,01	0,08	1,84	4,20	0,65
	0,29	4,80	0,06	3,68		
	0,18	3,63	0,21	3,56		
	0,19	4,67	0,50	4,88		
	0,04	2,20	0,17	2,31		
	0,16	2,63	0,65	4,77		
	0,17	4,53	0,08	2,23		
	0,08	3,67	0,18	2,97		
	0,01	4,03	0,17	3,02		
	0,13	4,77	0,28	2,84		
A6	0,15	3,87	0,54	7,32	6,40	0,51
	0,79	9,43	0,83	12,83		
	1,10	7,73	0,53	7,92		
	1,82	10,63	0,93	7,92		
	1,51	6,13	0,36	6,64		
	0,78	4,57	0,36	5,81		
	0,47	3,80	0,38	6,32		
	0,67	5,03	0,20	5,91		
	1,30	6,60	0,16	5,97		
	0,58	4,73	0,68	9,55		
A7	0,17	1,90	0,50	3,95	10,30	0,81
	0,19	2,90	0,05	3,52		
	0,45	9,37	0,28	4,92		
	0,39	3,87	0,36	3,80		
	0,51	5,30	0,85	11,15		
	0,50	5,90	0,20	3,68		
	0,47	5,10	0,07	2,00		
	0,49	6,33	0,64	9,12		
	0,65	8,87	0,04	1,56		
	0,61	1,80	0,31	3,95		

30% in the site A9, 175% in the site A5, and 300% in the site A8. The highest root biomass was observed in A8 with inoculation, and the lowest root biomass was observed in the sites A1, A11 and A3 without inoculation. From data in figure 2, without inoculation the shoot/root ratio varied from 14.4 to 2.0 g rDW plant⁻¹sDW plant⁻¹ in the sites A7 and A5, respectively, and with inoculation, from 7.0 to 3.9 g rDW plant⁻¹ sDW plant⁻¹ in the sites A12 and A2, respectively.

Efficiency in use of the rhizobial symbiosis and nitrogen accumulation

order to assess the effectiveness of the symbiosis for the

plant growth, the individual values of shoot and nodule biomass were plotted for each site where a significant regression was found between these two parameters (Table 3). Without inoculation, a regression was found only in the site A6 where the value of the regression-slope, i.e. the efficiency in use of the rhizobial symbiosis (EUSR) was 3.60±0.03 g sDWg⁻¹nDW. With inoculation three groups of EUSR values could be distinguished: 29.5±0.1 g sDWg⁻¹nDW in the sites A5 and A8; 10.4±0.2 g sDWg⁻¹nDW in the sites A6 and A7; 4.3±0.2 g sDWg⁻¹nDW in the sites A1 and A11.

In order to assess the nitrogen fixation per nodule biomass, the values of total nitrogen and nodule biomass per plant were plotted in Table 4. Total nitrogen varied from 1.0±0.06 mg N plant⁻¹ in the site A4 to 0.5± 0.03 mg

Table 3 Cont.

A8	0,13	1,93	0,17	8,19		
	0,11	2,57	0,29	17,02		
	0,75	4,17	0,33	21,50		
	0,25	3,30	0,30	8,00		
	0,41	1,20	0,70	26,68	23,70	0,79
	0,04	1,47	0,12	11,70		
	0,19	3,97	0,22	20,60		
	0,55	2,40	0,94	25,46		
	0,22	4,10	0,27	13,54		
	0,22	3,10	1,28	41,51		
A5	0,53	7,03	0,24	20,46		
	1,37	11,50	0,04	7,74		
	2,36	12,70	0,13	7,63		
	1,22	5,13	0,12	20,61		
	0,24	6,10	0,09	13,47		
	1,55	7,67	0,19	13,09	29,50	0,69
	0,44	15,27	0,15	11,30		
	1,57	4,13	0,77	29,79		
	0,35	7,83	0,06	7,97		
	1,42	8,17	0,08	6,44		

Table 4. Effect of rhizobial inoculation on total nitrogen content of cultivated faba bean in field sites. Data are means and SD of 20 replicates harvested at flowering stage without or with inoculation.

site	SDW c (g)	Ntot c(mg/pl)	NDWi (g)	Ntot i (mg/pl)
A9	0,505±0,26	0,54±0,16	0,291±0,16	1,07±0,26
A8	0,45±0,11	1,34±0,02	0,187±0,11	2,57±0,11
A4	0,33±0,01	6,19±0,16	0,48±0,16	2,62±0,01
A6	0,235±0,07	1,11±0,03	0,253±0,13	1,58±0,07
A2	0,18±0,11	1,11±0,23	0,251±0,14	1±0,11
A3	0,175±0,03	0,47±0,07	0,495±0,17	0,7±0,03
A1	0,165±0,4	0,89±0,15	0,254±0,15	0,7±0,03
A10	0,135±0,17	2,9±0,10	0,131±0,1	3,22±0,07
A7	0,125±0,13	0,95±0,06	0,33±0,06	1,79±0,03
A5	0,095±0,01	2,04±0,19	0,238±0,19	1,69±0,10
A12	0,075±0,03	0,75±0,02	0,17±0,15	1,38±0,16
A11	0,065±0,04	0,68±0,11	0,598±0,11	0,69±0,31

mean regression and correlation factor			
site	control plot	site	inoculated plot
A2		A5	
A3		A7	
A4	14; r2=0,7	A10	7;3; r2=0,99
A5		A11	
A8		A12	
A1		A1	
A6		A2	
A7		A3	
A9	36;r2=0,67	A4	0,14
A10		A6	
A11		A8	
A12		A9	

Nplant⁻¹ in the site A2 without inoculation, and from 1.7±0.1 mg N plant⁻¹ in the site A8 to 0.5±0.01 mg N plant⁻¹ in the

site A4 with inoculation (Table 4). Overall, the inoculation with local rhizobia strain increased the mean total N

Table 5. Effect of soil phosphorus on shoot dry weight of cultivated faba bean in field sites. Data are means and SD of 20 replicates harvested at flowering stage without or with inoculation

site	P Olsen	SDWc (g)	SDWi (g)
A9		91 0,505±0,26	0,291±0,16
A4		67 0,33±0,01	0,48±0,16
A2		55 0,18±0,11	0,251±0,14
A3		38 0,175±0,03	0,495±0,17
A10		63 0,135±0,17	0,131±0,1
A12		67 0,075±0,03	0,17±0,15
A1		89 0,254±0,15	0,165±0,4
A5		87 0,238±0,19	0,095±0,01
A6		83 0,253±0,13	0,235±0,07
A7		52 0,33±0,06	0,125±0,13
A8		94 0,187±0,11	0,45±0,11
A11		80 0,598±0,11	0,065±0,04

mean regression slope and correlation factor			
site	control plot	site	inoculated plot
A5	5,5; r2= 0,99	A1	9,5; r2=0,99
A6		A8	
A8		A7	
A9		A2	
A1		A3	
A2		A4	
A3		A5	
A4		A6	
A7		A9	
A10		A10	
A11		A11	
A12		A12	

Table 6. Effect of soil P on the efficiency in the use of rhizobial symbiosis of cultivated faba bean in field sites. Data are means and SD of 20 replicates harvested at flowering stage without or with inoculation

site	Psol Olsen	EUSR c (gNDW SDW)	EUSR i (gNDW SDW)
A1	80	0,6	4,3
A6	38	3,6	6,4
A7	52	8,39	10,3
A5	94	10,49	29,5
A11	97	5	6,2
A8	83	13,7	23,7
Mean regression slope		2,02	3,3
correlation factor		0,63	0,4

content in five sites.

Without inoculation, two significantly different regression-slopes of total nitrogen as a function of nodule biomass, i.e. the amount of N fixed per unit of nodule biomass, were found: 36.7±0.2 mg N fixed mg⁻¹ nDW in the sites A1, A6, A7, A9, A10, A11 and A12; 14.0±0.1 mg N fixed mg⁻¹ nDW in the sites A2, A3, A4, A5 and A8. The inoculation with Mat.11 induce a significant regression in the sites A5, A7, A10, A11 and A12 with mean value individual nodule N fixed of 7.3±0.02 mg N fixed mg⁻¹ nDW.

Available P in the rhizosphere soil and relationship with nodulation

In order to assess the effect of soil P availability on nodule biomass, the mean value of nodule biomass and soil Olsen P for each site were plotted in Table 5. However, soil P was so high that a critical P concentration for nodulation could not be found. Nevertheless for nodulation above 0.20±0.62 g nDWplant⁻¹, a significant regression was found in the sites A5, A6, A8 and A9 with mean regression-slope of 5.50±0.36

mg nDW mg⁻¹ soil P without inoculation, and 9.50±0.02 mg nDW mg⁻¹soil P with inoculation in the sites A1, A8 and A7.

The effect of soil P availability on EUSR (Table. 6) was studied in the sites A1, A5, A6, A7, A8 and A11 without inoculation and with inoculation where plant growth was significantly correlated with nodule biomass. Significant regression was found with inoculation with a mean regression slope of 2.05±0.1 g sDW-nDWmg⁻¹ soil P (r²=0.63) without inoculation and 3.3 gsDW-nDWmg⁻¹soil P (r²=0.44) in with inoculation treatment. The data in Table 6 showed that the EUSR decreased at P Olsen over than 90 mg P kg soil⁻¹except for the site A5 where the EUSR depended on another environmental condition.

DISCUSSION

The major result is that faba bean inoculation with Mateur rhizobia strain increased the nodule number and biomass in some sites comparing to the non-inoculated treatment. The increase of nodule biomass was generally associated with an enhancement of symbiosis efficiency through nodule growth and N₂ fixing at the expense of roots according to (Shultz et al.,1999) and (Voisin et al., 2003) and also at the expense of shoots during the vegetative stage (Voisin et al., 2003). According to this author, inoculation and P fertilization increases the nodular number and growth of the host plant. This agreement may be explained by the adaptation of Mateur strain to adverse local environments. The explanation for the inoculation failure in some field trials could be due to an inefficient population of indigenous faba bean rhizobia in the soils (Graham, 1981; Tang et al., 2001; Andrade and Hungria, 2002). Faba bean symbiotic nitrogen fixation provides an ecological and economical alternative to increase bean production but it depends on soil fertility and climate conditions.

In this work, the correlation of plant growth with nodulation as observed in many sites and with inoculation establishes that the symbiosis is the major determinant of faba bean growth. This contrast with the absence of any significant relation between these two parameters in the sites A5 and suggests that a major factor did prevent the symbiosis to contribute to plant growth in these sites. The efficiency in use of the rhizobial symbiosis of faba bean without inoculation suggests the potential of valuable rhizobial strains in some soils. The difference in adaptation of the symbiosis to various environments may be involved in the higher efficiency of Mat.11 in A5. The increase of the dry matter of plant biomass inoculated with rhizobia strains has been cited by many investigators (Sindhu et al., 2010; Zaidi et al., 2007; Hemissi et al., 2011).However, even though a lower nodule biomass was observed in some site a higher EUSR was reached along with an increased number of small-sized nodules. These nodules would probably have resulted from strong interspecific interactions with soil nutrients or it could be due to a change in the population. P

is among the most needed elements for crop production, and in heavily weathered acid soils, P is generally deficient and limits the potential input of SNF. Phosphorus deficiency reduces the number and biomass of nodules and their nitrogenase activity (Ribet and Drevon 1995; Vadez et al., 1996; Qiao et al., 2007) which contrary to the results observed in the studied soils where P does not affect the nodulation in some sites. The nodular growth would be modulated by the availability of P in the tissues Wall (2000). The higher P requirement for nodule growth may be explained by the requirement for plasmic membrane synthesis, in particular for the mitochondrias and the symbiosome in the infected cells. The competition for photosynthates between the root and the nodules might also be involved since the phosphorus deficiency more severely affected the distribution of biomass between nodules and root than between shoot and root for N₂-dependent soybean, (Cassman et al., 1980). This would be related to decreases in the number of leaves per plant and the unit leaf area as consequences of P deficiency inhibiting the leaf emergence and expansion.

Conclusion

The major result of our work is the large spatial and temporal variation of faba bean nodulation in the lower valley of Medjerda agro-ecosystem of Mateur, among experimental farmer's sites during the campaign 2013-2014, which has not been described previously at this scale to our knowledge.

Strain used to inoculate the multi-local field tests increased the amount of nitrogen fixed by symbiotic way and without additional nitrogen remains very useful in maintaining and restoring soil fertility compared to non inoculated cultures. It is also important to note that, in addition to the significant effect of inoculation on the nodulation it is also important to increase plant growth especially in the EUSR parameter in association with soil P. Further exploration of the interaction between the native rhizobia and locally used cultivars faba bean may be recommended for optimization of this inoculant-biotechnology.

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Conflict of interests

The authors declare that they have no conflicting interests.

REFERENCES

- Abel S, Ticconi CA, and Delatorre CA (2002). Phosphate sensing in higher plants, *Plant Physiol.* 115: 1-8.
- Andrade S, Hungria M (2002). Maximizing the contribution of biological nitrogen fixation in tropical legume crops. In: Finan, T.M., O'Brian, M.R., Layzell, D.B., Vessey, J.K., Newton, W.E. (Eds.), *Nitrogen Fixation Global Perspectives*. CABIO Publishing, London, pp. 341-345
- Camila MP, Lazara C (2004). Nitrogen-fixing and vesicular-arbuscular mycorrhizal symbiosis in some tropical legume trees of tribe *Mimoseae* *Forest. Ecol Man.* 196: 275-285.
- Cassman KG, Whitney AS, Stockinger KR (1980). Root growth and dry matter distribution of soybean as affected by phosphorus stress, nodulation and nitrogen source. *Crop Sci.*, 20: 239-244.
- Graham PH (1981). Some problems in nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris*. *Field Cr Res.* 4: 93-112.
- Graham PH (2008). Ecology of the root nodule bacteria of legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (Eds) *Nitrogen fixing leguminous symbiosis*, Springer, Dordrecht. The Netherland, pp 23-58
- Graham PH, Vance CP (2003). Legumes: importance and constraints to greater utilization. *Plant Physiol.* 131: 872-877.
- Hemissi I, Mabrouk Y, Abdi N, Bouraoui M, Saidi M and Sifi B (2011). Effects of some *Rhizobium* strains on chickpea growth and biological control of *Rhizoctonia solani*. *Afr J Micr Res.* 5(24): 4080-4090.
- Israel DW (1987). Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant Physiol.* 84: 835-840.
- Lazali M, and Drevon JJ (2014). The nodule conductance to O₂ diffusion increases with phytase activity in N₂-fixing *Phaseolus vulgaris* L. *Plant Physiol Biochem.* 80:53-59.
- Lazali M, Bargaz A, Carlsson G, Ounane SM, and Drevon JJ (2014). Discrimination against ¹⁵N among recombinant inbred lines of *Phaseolus vulgaris* L. contrasting in phosphorus use efficiency for nitrogen fixation. *J Pl Physiol.* 171:199-204.
- Lazali M, Brahimi S, Merabet C, Latati M, Benadis C, Maougal RT, Blavet D, Drevon, JJ and Ounane SM (2016). Nodular diagnosis of contrasting recombinant inbred lines of *Phaseolus vulgaris* in multi-local field tests under Mediterranean climate. *Europ J of Soil Biol.* 73:100-107.
- Ministry of agriculture (2007). Tunisia.
- O'Hara GW, Boonkerd N, and Dilworth MJ (1988). Mineral constraints to nitrogen fixation. *Plant Soil.* 108: 93-110.
- Olsen SR, Cole CV, Watanabe FS, and Dean LA (1954). Estimation of Available phosphorus in Soil by Extraction with Sodium Bicarbonate; Circular 939.USDA, Washington DC, USA, pp. p19.
- Pereira PAA, Bliss FA (1989). Selection of common bean (*Phaseolus vulgaris* L.) for N₂ fixation at different supplies of available phosphorus under field and environmentally-controlled conditions. *Plant Soil.* 115: 75-82.
- QiaoY, Tang C, Han XZ, Miao SJ (2007). Phosphorus deficiency delays the onset of nodule function in soybean (*Glycine max* Murr.). *J Pl Nutr.* 30: 1341- 1353
- Ribet J, Drevon JJ (1995). Phosphorus deficiency increases the acetylene- induced decline in nitrogenase activity in soybean (*Glycine max* L. Merr.). *J Ex Bot.* 46: 1479- 1486.
- Shultze S, Keatinge, JDH, Wells GJ (1999). Productivity and residual effects of legumes in rice-based cropping systems in a warm-temperate environment. II. Residual effects on rice. *Field Cr Res.* 61(1): 37-49
- Sindhu S, Dua S, Verma MK, Khandewal A (2010). Growth Promotion of Legumes by Inoculation of Rhizosphere Bacteria. M.S.Khan et al. (eds.). *Microbes for Legume Improvement*. Springer-Verlag Wien. Germany.
- Tang C, Hinsinger P, Jaillard B, Rengel Z, Drevon JJ (2001). Effect of phosphorus deficiency on the growth, symbiotic N₂ fixation and proton release by two bean (*Phaseolus vulgaris*) genotypes. *Agric.* 21: 683-689.
- Vadez V, Rodier F, Payre H, Drevon JJ (1996). Nodule permeability to O₂ and nitrogenase linked respiration in bean genotypes varying in the tolerance of N₂ fixation to P deficiency. *Plant Physiol Biochem.* 34: 871-878.
- Vincent JM (1970). The cultivation, isolation and maintenance of rhizobia. In a manual for the practical study of root nodule. In: Vincent, J.M. (Ed.) Oxford: Blackwell Scientific Publications, p: 1-13.
- Voisin AS, Salon C, Warembourg FR (2003). Seasonal patterns of ¹³C partitioning between shoot and nodulated roots of N₂-or nitrate fed- *Pisum sativum* (L). *Annals of Botany.* 91: 539-546.
- Wall LG (2000). Actinorhizal symbioses, J. *Plant Growth Regulation.* 19: 167-182.
- Zaidi A, Khan MS (2007). Stimulatory effects of dual inoculation with phosphate solubilizing microorganisms and arbuscular mycorrhizal fungus on chickpea. *Aust J Ex Agr.* 47: 1016-1022.