J. Mater. Environ. Sci. 6 (4) (2015) 997-1003
ISSN : 2028-2508
CODEN: JMESCN

Growth and nodulation in faba bean-rhizobia symbiosis under different soil phosphorus levels: acid phosphatase and phosphorus deficiency tolerance

L. Boudanga[▲1], M. Farissi[▲1], A. Bouizgaren[2], C. Ghoulam*[1]

[1]*Unit of Plant Biotechnology and Symbiosis Agro-physiology, Faculty of Sciences and Techniques, PO. Box 549, Gueliz, 40000 Marrakesh, Morocco*

[2]*Unit of Plant Breeding, National Institute for Agronomic Research (INRA), PO. Box 533, Gueliz 40000, Marrakesh, Morocco.*

Received 25 Aug 2014, Revised 3 Dec 2014, Accepted 3 Dec 2014
[*]Corresponding Author. E-mail: ghoulam@fstg-marrakech.ac.ma ; Tel: (+212(0)668730172)

[▲] These authors contributed equally to this work.

Abstract

The symbiosis faba bean-rhizobia behaviors under different soil phosphorus levels were studied. The plants were grown under symbiotic nitrogen fixation without fertilizers application. At flowering and maturity stages, ten plants per site were harvested and analyzed for their growth and nodulation, phosphorus and nitrogen contents. The acid phosphatases activity was analyzed in nodules and rhizospheic soils. Results showed that the nodular and shoot dry biomass were associated to the richness of the soil in available phosphorus. The highest and significant dry biomass was noted in plants of site 2 having a highest level in soil available phosphorus (85 and 0.66 g .plant^{-1} for shoots and nodules respectively) while the lowest values were noted with the plants of site 3 (43.75 and 0.43 g .plant^{-1} for shoots and nodules) showing the smallest soil available phosphorus. The P contents were associated with the soil P availability. The highest values were noted in plants of site 2 (11.46 mg P. g DW^{-1} for shoots and 19.50 mg P. g DW^{-1} for nodules). The amounts of nitrogen accumulated ranged from 2.51 to 4.37 mg . g DW^{-1} in shoots and from 3.36 to 6.01 mg . g DW^{-1} in nodules. The soil phosphorus level influenced the potassium sorption. Low levels of soil available phosphorus accentuated the enzymatic activity of acid phosphatase in nodules and rhizospheric soil. The increase in acid phosphatases activity could be an adaptation mechanism developed by faba bean plants for tolerance to phosphorus deficiency stress.

Keywords: Faba bean, Growth, Nodulation, Phosphorus, Acid phosphatase

Introduction

Legumes are a major source of protein and vegetable oils. They are widely cultivated throughout the world [1]. In Mediterranean area, these plants occupy an important place due to their agro-economic and environmental interests. Indeed, leguminous plants have a very favorable influence on soil fertility by contributing to the incorporation of nitrogen in ecosystems offering thus beneficial, ecological and economical impacts, helping to reduce or limit the use of chemical fertilizers by nitrogen-fixing symbiosis involving rhizobial strains [2]; [3]. However, in arid and semi arid areas, legume-rhizobia symbiosis is negatively affected by many environmental constraints, such as water deficit, salinity, temperature variations and soil deficiency in inorganic nutrients particularly available phosphorus for plants [4]; [5]; [6]; [7]; [8]; [9]. Indeed phosphorus (P) is a key nutrient limiting the productivity of legumes [10]. The majority of the phosphorus contained in the soil is in inorganic and organic complex forms. These forms are not directly usable by the plants [11]. Indeed, the high reactivity of P with iron, aluminum and calcium, to form insoluble compounds, reduces its mobility in the soil solution. These reactions provoked a very low P availability and low efficiency of phosphate fertilizers used by plants [12].

Nutritional deficiency related to soil P low availability is the major factor limiting symbiotic nitrogen fixation process [10], root growth, the process of photosynthesis, translocation of sugars and other functions [12].

Phosphorus deficiency also affects the growth of rhizobia [4] and reduces the growth of nodules [10];[13]. In addition to phosphorus demand of the host plant, nodules require larger amounts of P than other plant tissues [14].
Morphological responses to phosphorus deficiency involve altering root architecture, mainly by a decrease of the primary root growth and increased lateral root number and the formation of root hairs [15]; [16]. Physiological and biochemical responses include changes in carbon metabolism, synthesis and secretion of phosphatases and phytases, exudation of organic acids (citrate, malate), and improving the expression of high affinity phosphate transporter [17]; [15]; [16]. The phosphatases are enzymes involved in the recovery of phosphate trapped in organic molecules allowing thus the improvement of symbiosis P nutrition.
In this context, the present work aims to evaluate the behavior of faba bean-rhizobia symbiosis under phosphorus deficiency through monitoring the plots with different levels of soil available Pi. The evaluation focused the growth and nodulation and some physiological and biochemical properties associated with phosphorus deficiency tolerance as phosphatase acid (APase) activity.

2. Materials and methods

2.1. Site description and growth conditions

The present study was carried out at four small farmers' fields in a semi arid zone of Haouz area (Tamazouzt) at the region of Marrakesh (sub-centre of Morocco) with the following geographical coordinates: Altitude: 631 m - Latitude: 31°38'08''.2, North-Longitude: 7°45'41''West. The soil available P was determined for the plot prior to culture installation. The experiments were conducted during 2011. The trial's management was the same as applied locally and plants were grown under symbiotic nitrogen fixation without chemical fertilizers application. The plants were irrigated once a week using a gravity irrigation system. The evaluation of the plots was performed by sampling 10 plants per site and soil samples. In the laboratory, the samples were subjected to different analyzes.

2.2. Soil Analyses

Soils characterization was based on measuring of many physical and chemical parameters. The soil pH was determined with a pH meter using a portion of 10 g of soil and 25 ml of distilled water for pH water. For pH KCl, 25 ml KCl (1 $mol.L^{-1}$) and 25 ml of distilled water were used. The electrical conductivity (EC) was determined with a conductivimeter using an aqueous extract of soil (20 g of soil sample in 100 ml of distilled water). Total P was determined after igniting air dried soil samples at 550 °C for 4 h and dissolving the ashed samples in concentrated HCl. The soil available P (Olsen P) to plants was determined after extraction in 0.5 M $NaHCO_3$ [18]. Available and total P were analyzed by the molybdate blue method by reading the absorbance at 820 nm after color development at 100 °C for 10 min [19]. Sodium (Na^+) and potassium contents (K^+) were determined using a flame spectrophotometer Jenway type [20]. Soil physicochemical properties of all sites studies were presented in Table 2.

2.3. Plant and nodular dry weights

Ten plants were sampled from each site and separated to shoots and nodulated roots. Roots and nodules were carefully separated from rhizospheric soil, washed through a sieve and then the nodules were detached. This allows to retrieve as maximum as possible of nodule and root biomass from the detached rhizospheric soil. Shoots and nodules were dried at 70 °C for 3 days to determine their dry weights and thereafter dry samples were ground to enable determination of P and N contents.

2.4. APase activity in rhizosphere soils

The nodulated roots were dug to 20 cm depth and the adhered soil layers (~2 mm) were collected and designated as rhizosphere soil [6]. All the soil samples were first sieved (<2 mm) and immediately stored at 4 °C until further analyses for activities of APase.
Soil APase activity was determined using pNPP as an orthophosphate monoester analogue substrate [21]. Briefly, 125 mg of each soil sample was placed in a 1.5 mL Eppendorf flask, 500 µL of 0.2 M sodium acetate buffer pH 5.6 and 125 µL of 10 mM pNPP, were added and the flask was swirled for a few seconds. After 30 min of incubation at 30 °C, 125 µL of 0.5 M $CaCl_2$ and 500 µL of 1M NaOH were added, and swirled the flask to stop the reaction. The soil suspension was centrifuged for 10 min at 5000 g to avoid the interference of possible precipitates and absorbance was measured at 405 nm against the reagent blank and p-nitrophenol content determined by reference to a standard curve. The protein concentrations were determined by Bradford method using the bovine serum albumin as a standard.

2.5. APase Activity in Nodules

100 mg nodular fresh weight of each plant was carefully detached at late flowering stage and immediately frozen at -20 °C. Each sample of nodule was ground. APase was extracted and assayed accordingly to the method described by Araújo et al. [22].

2.6. Phosphorus and nitrogen contents

Shoot and nodular P contents were determined using the molybdate blue method according to Murphy et al. (1962) [19].The ashed dried subsamples at 550 °C were dissolved in 3 mL of concentrated HCl and absorbance was measured at 820 nm. For N determination, 0.5 g of subsamples were used and analyzed by the Kjeldahl method.

2.7. Potassium contents

For Potassium (K^+) contents determination, samples (0.5 g) of dried shoots and nodules were ashed in a furnace for 6 h at 500 ºC. The ash was dissolved in chloride acid. This solution was diluted with distilled water and filtered on Whatman paper. The K^+ contents were determined by flame emission photometry [20].

2.8 Statistical analysis

Statistical analysis was performed using SPSS (10.0) software. It concerned a two-way analysis of variance and Student-Newman- Keuls grouping test.

3. Results

3.1. Chemical and physical properties of the soils used in the study

Soil sites sampled in this study cover a wide range of properties towards their chemical composition and phosphorus contents (table 2). The soil pH of sites studied was approximately similar. The values recorded indicated that the pH was slightly alkaline. The electrical conductivities of all parcels studied were lower than 4 ms .cm^{-1} indicating that the soils of sites studied were not saline.

The available P contents in soils studied showed a large variation depending on the site. We noted a correlation between soil pH and its content in available P. The high levels were recorded for soils of site 2 (0.95 mg. kg $soil^{-1}$) and the lowest contents were noted in site 3 (0.59 mg.kg $soil^{-1}$). For total phosphorus, its content was significantly higher in sites 1 and 2 compared to the remaining sites.

Table 1: Some determined soil physical and chemical properties of the studied sites.

Sites Studied	pH_{Water}	pH_{KCl}	EC $ms.cm^{-1}$	P_{Total} $g.Kg\ sol^{-1}$	P_{Olsen} $mg.Kg\ sol^{-1}$	Na^+ $g.Kg\ sol^{-1}$	K^+ $g.Kg\ sol^{-1}$
Site 1	7.82	7.34	0.33	1.37	0.73	0.93	1.19
Site 2	7.77	7.07	0.67	1.35	0.95	1.04	1.57
Site 3	8.22	7.82	0.16	1.14	0.59	0.84	0.85
Site 4	7.86	7.22	0.26	1.20	0.62	0.95	1.08

3.2. Growth and Nodulation

The shoot dry biomass varied between 43.75 and 85 g .$plant^{-1}$ (Figure 1). The plants of site 2 showed the highest and significant ($P < 0.001$) dry biomass (85 g . $plant^{-1}$) while the plants of site 3 showed the lowest dry biomass (43.75 g .$plant^{-1}$). These results were associated with a high level of site 2 in available P and a high rate of nitrogen released by the previous crop.

For nodular dry biomass, there was also a very highly significant variation ($P <0.001$) between different sites, with a maximum of 0.66 g .$plant^{-1}$ for nodules from site 2. Plants of site 3 showed the lowest nodular dry biomass (0.43 g .$plant^{-1}$).

3.3. Shoot and nodular total P contents

Data in figure 2 indicated that phosphorus concentrations in different organs of faba bean plants showed significant differences ($P <0.001$) between the sites studied. For shoots, total P levels ranged between 6.14 and 11.46 mg P. g DW^{-1}. The maximum values were recorded in plants of site 2 (11.46 mg P. g DW^{-1}) where the soil is rich in available P (Table 2), followed by those of site 1 (10.07 mg P. g DW^{-1}). The lowest total P content has been recorded in plants of site 3 (6.14 mg P. g DW^{-1}). We note that the soil of this site showed low levels of available P.

Faba bean nodules accumulated more phosphorus than the shoots with the highest contents of 19.50 mg P. g DW^{-1} recorded in plants of site 2 and an average level between 12 and 13 mg P. g DW^{-1} for plants of sites 4 and 1. Low values of 9.41 mg P. g DW^{-1} were recorded in plants of site 3.

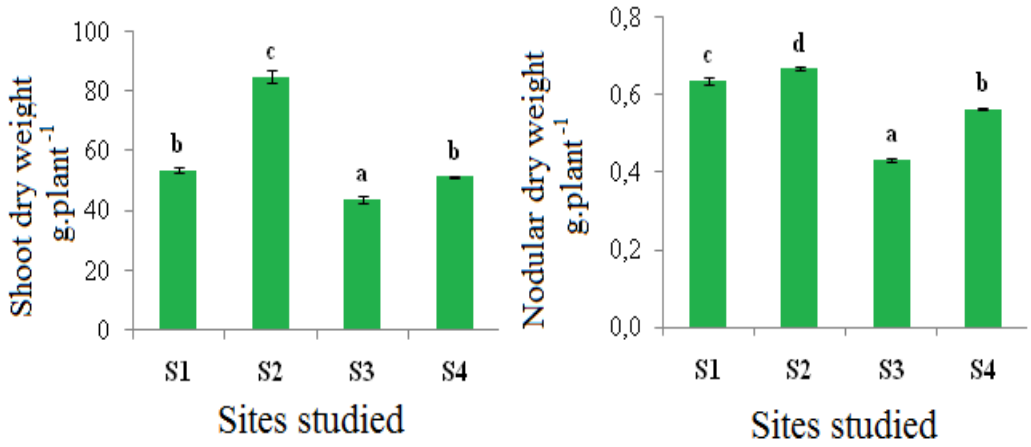

Figure 1: Shoot and nodular dry weights in faba bean-rhizobia symbiosis grown under different soil available P levels in sites studied (S1, S2, S3 and S4). Results are means of ten replicates and Bars are standard errors.

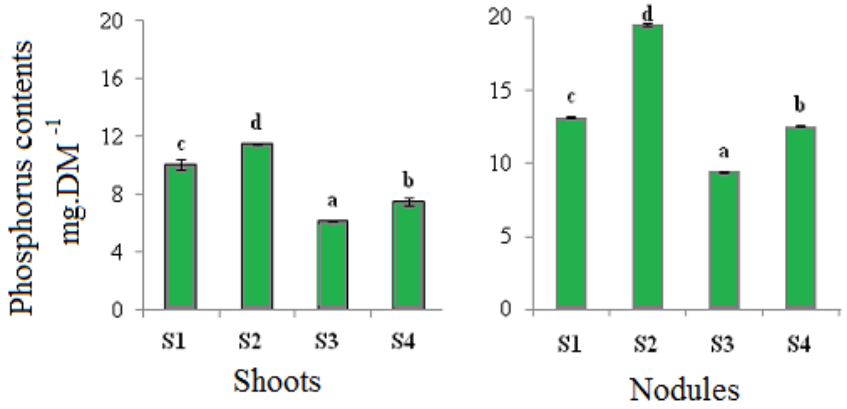

Figure 2: Shoot and nodular phosphorus contents in faba bean-rhizobia symbiosis grown under different available soil P levels in sites studied (S1, S2, S3 and S4). Results are means of six replicates and Bars are standard errors.

3.4. Shoot and nodular total N contents

The amounts of nitrogen accumulated in faba bean plants ranged from 2.51 to 4.37 mg . g DW^{-1} in shoots and from 3.36 to 6.01 mg . g DW^{-1} in nodules (figure 3). The planting of faba bean on a soil rich in P available as that of site 2, led the accumulation of high nitrogen contents (4.37 mg . g DW^{-1} for shoots 6.01 mg . g DW^{-1} for nodules). For the site 3, poor in available P, the levels of total N accumulated not exceeded 2.51 mg mg . g DW^{-1} for shoots. The similar behavior was recorded in the corresponding nodules. The intermediate values were noted with the plants of sites 1 and 4.

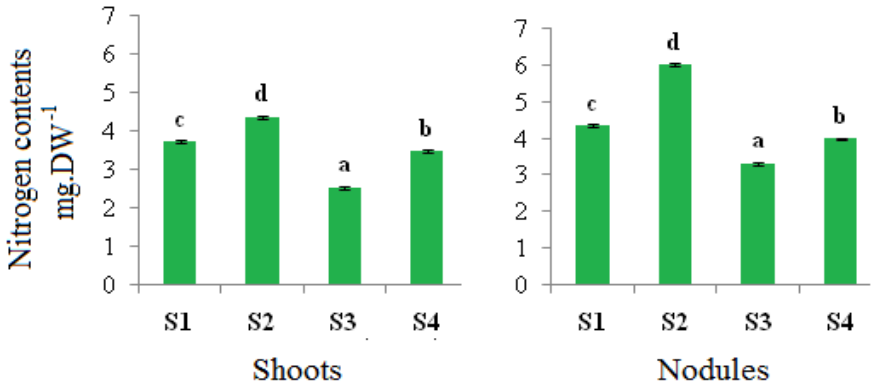

Figure 3: Shoot and nodular nitrogen contents in faba bean-rhizobia symbiosis grown under different soil P levels in sites studied (S1, S2, S3 and S4). Results are means of six replicates and Bars are standard errors.

3.5. Nodular APase activity

The results in figure 4 showed that the site effect on the variation of the nodular APase activity of plants sampled is highly significant (P <0.001). This APase activity ranged from a lowest value of 0.4 µg PNP. min^{-1}. g $protein^{-1}$, recorded in nodules from site 2 characterized by the highest soil available P, to a highest value of 0. 52 µg PNP. min^{-1}. g $protein^{-1}$ noted with nodules of site 3 characterized by its low content in soil available P. The moderate APase activities were observed in nodules of the remaining sites.

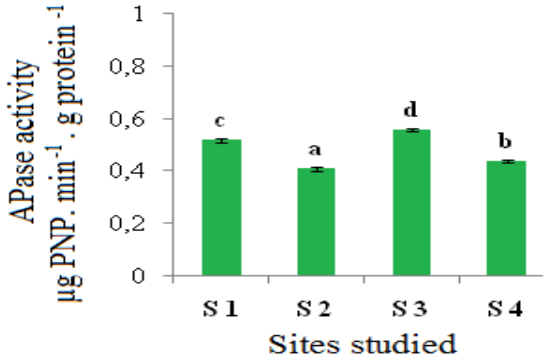

Figure 4: Nodular acid phosphatase activity in faba bean-rhizobia symbiosis grown under different soil P levels in sites studied (S1, S2, S3 and S4). Results are means of six replicates and Bars are standard errors.

3.6. Rhizospheric soil APase activity

The enzymatic activity of acid phosphatase in the rhizosphere of plants sampled showed significant variations between the sites studied (Figure 5). The site 3 which was characterized by lowest level of soil available P showed the highest rhizosphere APase activity (0.79 µg PNP. min^{-1}. g sol^{-1}). The rhizosphere APase activity in sites 1 and 4 were not significantly different (P>0.05). The rhizosphere soil of site 2 characterized by a high content of available P recorded the lowest value of APase (0.55 µg PNP. min^{-1}. g sol^{-1}).

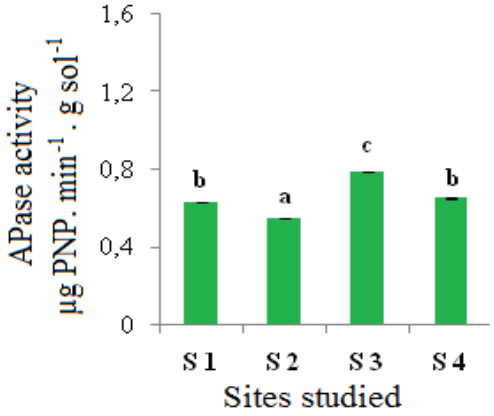

Figure 5: Rhizospheric soil acid phosphatase activity in faba bean-rhizobia symbiosis grown under different soil P levels in sites studied (S1, S2, S3 and S4). Results are means of six replicates and Bars are standard errors.

3.7. Potassium contents

The potassium content ranged from 0.36 to 0.78 mg.g DW^{-1} for faba bean nodules and for 0.88 to 1.17 mg . g DW^{-1} for shoots (Figure 6). The nodules from site 2 showed a higher content of potassium (0.78 mg. g DW^{-1}), whereas those of the site 3 showed the lowest content (0.36 mg .g DW^{-1}). For shoots, plants of site 3, with the lowest content of soil available P, presented the lowest contents (0.88 mg. g DW^{-1}). However, those of the site 2 having a highest soil available P, showed the highest content of potassium (1.17 mg.g DW^{-1}).

4. Discussion

The evaluation results of faba bean plants and soils sampled showed a net change in nodular and shoot dry weight depending on the site. Similarly, for the contents of soil available P, we noted a significant variation between the sites. For all plots sampled, we found a positive correlation between dry biomass and soil available P. Indeed, the plants of site 2 having the highest content of sol available P showed the highest dry biomass while the lowest dry biomass where observed in sites with low soil available P particularly site 3.

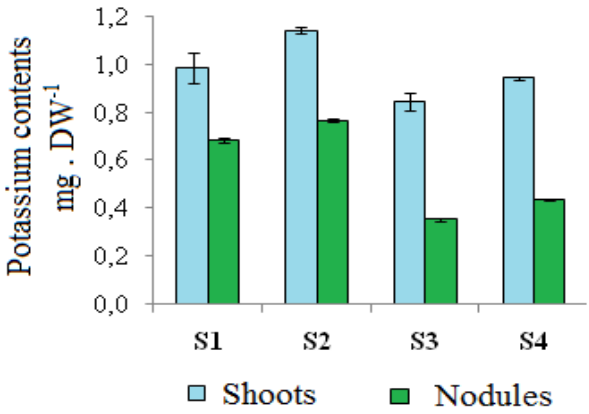

Figure 6: Shoot and nodular potassium contents in faba bean-rhizobia symbiosis grown under different soil P levels in sites studied (S1, S2, S3 and S4). Results are means of six replicates and Bars are standard errors.

The plants were grown under symbiotic nitrogen fixation and the variations observed in growth and nodulation could be attributed mainly to the effect of available phosphorus in the soil. Ghoulam et al. (2007) [23] and Bargaz et al. (2012) [6] have reported the similar results with common bean in the same region of the Haouz. They attributed the variations of biomass to the effect of soil available phosphorus. Togay (2008) [24] found that phosphorus significantly increased the shoot dry weight of faba bean, lentils and soybean. In the same sense, Siddiqui (2007) [25] reported that the effect of phosphorus was significant on the dry weight of legumes. The phosphorus sorption capacity was strongly related to the production of dry biomass [26]. A phosphorus deficiency has a depressive effect on nodulation, leaf surface, shoot and root growth of legumes [24]; [27]. In the same way, Sulieman et al. (2009) [26] documented that the legume growth has increased more rapidly with the increase of phosphorus in the soil solution.

Our results also showed that the concentrations of P in shoots and nodules were generally higher for plants having high dry biomass and low for those showing low dry biomass proving consequently the positive effect of P on the plant growth and nodulation. In this sense, Sulieman (2009) [26] showed that with the exception of nitrogen, no other nutrient has been essential to the growth of plants that the phosphorus. A deficiency in this element has double impact because it can prevent the sorption of other nutrients by plants [27]. Indeed, through our results, we observed a positive correlation between of shoot and nodular potassium contents with the richness of the soil in available phosphorus. In the same sense, we found a positive correlation between the nitrogen content of nodules and soil available phosphorus. This finding confirms the work reported by other researchers [28]; [29].

Our data showed that the nodular and rhizospheric soil APase activities increased with the soil phosphorus deficiency. Indeed, we noticed the highest APase activity in the site 3 which showed the lowest value in terms of available P compared to other sites. Mandri et al. (2012) [13] and Bargaz et al., (2012) [6] concluded that phosphorus deficiency increases the activity of phosphatases in the nodules of *Phaseolus vulgaris*. This could be an adaptation mechanism developed by leguminous plant to overcome phosphorus deficiency stress. Increased phosphatase activity under phosphorus deficiency appears to be correlated with an increase in the expression of genes coding for phosphatases [30]. However, biochemical and molecular studies strongly suggest that the secretion of phosphatase is an integral part of the mechanisms of plant response to low soil P availability [30]. In general, in agricultural soils, the solubilization of inorganic phosphate is closely associated to the activity of soil microorganisms including rhizobia [31]; [32]; [33]. Indeed, rhizobia can secrete substances in their culture media during their growth, allowing to inorganic phosphate solubilization [4].

Conclusion

We conclude that the nodular and shoot dry biomass of faba bean were associated to the richness of the soil in available phosphorus. The nodular and shoot P contents were associated with the soil P level. The potassium sorption was influenced by the content of phosphorus in the soil. Low levels of soil available phosphorus accentuated the enzymatic activity of acid phosphatases in nodules and rhizospheric soil. The increase in acid phosphatases activity could be an adaptation mechanism developed by faba bean plants to overcome phosphorus deficiency stress.

Acknowledgements-This work was financially supported by FABATROPIMED project funded by Agropolis Foundation. The authors thank the famers of Tamazouzt region who have made available their materials and their trials for this study.

References

1. Graham P.H.,Vance C.P., *Plant Physiol.* 131 (2003) 872-877.
2. Faghire M., Bargaz A., Farissi M., Palma F., Mandri B., Lluch C., Tejera García N. A., Herrera-Cervera J. A., Oufdou K., Ghoulam C., *Symbiosis* 55 (2011) 69-75.
3. Latrach L., Farissi M., Mouradi M., Makoudi B., Bouizgaren A., Ghoulam C. *Turk. J. Agric. For.* 38 (2014) 320-326.
4. Farissi M., Bouizgaren A., Faissal A., Faghire M., Ghoulam C. *PJAR*. 2 (2014b) 9-19.
5. El Harrak N., Elazhar F., Zdeg A., Zouhri N., Elazhar M., El midaoui A., *Mor. J. Chem*. 2 (2014) 438-442.
6. Bargaz A., Faghire M., Abdi N., Farissi M., Sifi B., Drevon J.J., Cherkaoui M.I., Ghoulam C., *Agriculture* 2 (2012)139-153.
7. Farissi M., Bouizgaren A., Faghire M., Bargaz A., Ghoulam C., *Turkish J. Bot*. 37 (2013a) 1166-1175.
8. Farissi M., Ghoulam C., Bouizgaren A., *Fourrages* 216 (2013b) 329-332.
9. Farissi M., Faghire M., Bouizgaren A., Bargaz A., Makoudi B., Ghoulam C., *J. Agr. Sci. Tech*. 16 (2014a) 301-314.
10. Drevon J.J., Sifi B., La fixation symbiotique de l'azote et développement durable dans le bassin méditerranéen. Edition INRA-France (2003) 418p.
11. Turner B.L., Organic phosphorus transfer from terrestrial to aquatic environments. In: Turner B.L., Frossard E. and Baldwin D.S. (eds.), Organic phosphorus in the environment, CAB International, Wallingford, (2005) 269-294
12. Zarrin F., Zia M., Chaudhary M .F., *Pak. J. Bot*. 39 (2007) 255-264.
13. Mandri B ., Drevon JJ., Oufdou K., Plassard C., Payre H., Bargaz A., Ghoulam C., *J. Plant Nutr*. 35 (2012) 1477-1490.
14. Chagas E., Araujo A.P., Alves B.J.R., Teixeira M.G., *Rev. Bras. Ciênc. Solo* 34 (2010)1093-1101.
15. Vance C.P., Uhde-Stone C., Allan D.L., *New Phytol*. 157 (2003) 423-447.
16. Shulaev V., Cortes D., Miller G., Mittler R., *Physiol. Plant* 132 (2008) 199-208.
17. Smith F.W., *Plant Soil* 232 (2001) 109-118.
18. Olsen S.R., Cole C.V., Watanabe F.S., Dean L.A., Estimation of Available Phosphorus in Soil by Extraction with Sodium Bicarbonate; Circular 939; USDA: Washington, DC, USA, (1954) 19p.
19. Murphy J., Riley J.P., *Acta Anal. Chim*. 27(1962) 31-36.
20. Ghoulam C., Foursy A., Fares K., *Environ. Exp. Bot*. 47 (2002) 39-50.
21. Tabatabai M.A., Soil enzymes. In Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties; Soil Science Society of America: Madison, WI, USA, (1994) 775-833.
22. Araújo A.P., Plassard C., Drevon J.J., *Plant Soil* 312 (2008) 129-138.
23. Ghoulam C., Raguibi H., Mesbah H., Mandri B., Oufdou K., Drevon J. J. Assessment of Common bean/rhizobia symbiosis performance under different soil phosphorus levels. Second International rhizosphere Conference, August 26-31, Montpellier (2007).
24. Togay Y.,Togay N., DoganY., *Afr. J. Biotechnol*.7(2008)1256-1260.
25. Siddiqui H., *Int. J. Sustain. Crop Prod*. 2 (2007) 21-24.
26. Sulieman S.A., Abdalla M.A., Omer E.A., Hago T.E.M., *Aust. J. Basic & Appl. Sci*. 3 (2009) 2598-2606.
27. Mengel K., Kirkby E.A., Principles of Plant Nutrition (5th edition) Kluwer Academic Publishers, Dordrecht, The Neitherlands (2001).
28. Schulze J., Temple G., Temple S., Beschow H., Vance C.P., *Annals Bot.* 98 (2006) 731-740.
29. Tsvetkova G.E., Georgiev G.I., *J. Plant Nutr*. 30 (2007) 2129-2140.
30. Bargaz A., Lazali M., Amenc L., Abadie J., Ghoulam C., Farissi M., Faghire M., Drevon J.J., *Planta* 238 (2013) 107-19
31. Richardson A. E., *Aust. J. Plant Physiol*. 28 (2001) 897-906.
32. Sundara B., Natarajan V., Hari K., *Field Crops Res*. 77 (2002) 43-49.
33. Bouajila K., Sanaa M., *J. Mater. Environ. Sci.* 2 (2011) 485-490.