Carpathian Journal of Earth and Environmental Sciences August 2022, Vol. 17, No. 2, p. 287 – 296; DOI:10.26471/cjees/2022/017/222

IMPORTANCE OF BIOFFERTLIZATION VIA RHIZOBIA STRAIN ON PEA (PISUM SATIVUM L.) PRODUCTION IN A STRESSED EDAPHO-CLIMATIC ENVIRONMENT, IN TUNISIA

Khadija OUERGHI[1,2], Najla LASSOUED[2,3], Essaied BILAL[4*] & Bouaziz SIFI[1]

[1]*National Agricultural Research Institute of Tunisia, Hédi Karray Street, 1004 ElMenzah, Tunisia.*
[2]*National Agronomic Institute of Tunisia, 1082 Mahragène Tunis, Tunisia*
[3]*National Institute for Rural Engineering' research, Water and Forestry, BP10, 2080 Ariana, Tunis*
[4]*Ecole Nationale Supérieure des Mines de Saint Etienne, CNRS UMR 5600, F42023 Saint Etienne, France ; bilalessaid@gmail.com*

Abstract: Pea (Pisum sativum L.) is a nutritious leguminous rich source of protein. It plays an important role in crop rotation and contributes efficiently to soil fertility. Despite these nutritional, symbiotic and agronomic characteristics, pea cultivation areas are in regression and yield remain low. Abiotic and biotic constraints as well as the optimization of symbiotic nitrogen fixation are the main factors affecting the pea crop development. This work aimed to study the effects of inoculation with rhizobia having high potential solubilization of inorganic phosphorus on pea production under edapho-climatic stress conditions. Inoculation with rhizobia and phosphorus fertilization trials were performed in vitro at the laboratory and in vivo in fields at two experimental stations for three successive crop seasons characterized by contrasted precipitations regimes. The inoculation with both selected strains of Rhizobium (TAC and 12362) increased significantly the biomass nodular and improved the phosphorus content and phosphorus use efficiency (PUE) of pea plants. The phosphorus application induced symbiosis efficiency and increased nodulation and biomass production of pea. Regardless of the treatment, a highly significant effect of the season and site on the variability of pea production was recorded. Inoculation with rhizobia could substitute nitrogen and phosphorus chemical fertilizers for economic, ecological and sustainable agriculture.

Key words: Pisum sativum, Rhizobium, solubilization, phosphorus, biomass, ecological farming, PUE.

1. INTRODUCTION

Phosphorus is one of the major crucial elements for the growth and development of plants (Rosas et al., 2006). Plants absorb phosphates from the soil solution under two main forms of inorganic anions, which are $H_2PO_4^-$ (under acidic conditions) and HPO_4^{2-} (under alkaline conditions) (Benadis et al., 2014). Phosphorus is a little mobile element in the soil (Park et al., 2011). However, its availability in soil is low (Marschner et al., 2011) constituting a limiting factor for the growth of plants (L'taief et al., 2011; Hmissi et al., 2015; Kouki et al., 2016); for this reason, it was necessary to apply phosphate fertilizers (Zaman-Allah et al., 2006; Abdi et al., 2014). In this context, several researches have been carried out to better understand phosphorus availability mechanisms in soil (Attar et al., 2012; Latati et al., 2014; Tajini & Drevon, 2014) and phosphorus use efficiency (PUE) in order to sustain food production and minimize environmental impact (Sandana & Pinochet, 2014). In agricultural soils, the dissolution of inorganic phosphates is closely related to the activity of microorganisms in soil which is more intense in the rhizosphere owing to the presence of a large number of microorganisms in the soil (bacteria, fungi and algae) (Richardson, 2001; Oehl et al., 2001; Sundara et al., 2002; Hmissi et al., 2015). Although the solubilization of phosphates is associated with a medium pH decreasing, certain chemoautotrophic bacterial strains derive their growth energy from the oxidation of certain chemical elements with the production of acids (Hinsinger, 2001). The bacterial strains of the genus Rhizobium, Bradyrhizobium, Sinorhizobium and Pseudomonas are known for their high phosphorus solubilization potential (Rosas et al., 2006; Schulze et al., 2011).

In the Mediterranean regions, several studies were discussed and focused on the genus Rhizobium in symbiosis with several legumes such as beans (Bargaz et al., 2012; Abdi et al., 2014; Kouki et al., 2016) and chickpea (L'taief et al., 2009; Hmissi et al., 2015).

However, the production of pulses in these regions is largely dependent on the availability of water and nutritive elements such as nitrogen and phosphorus (Sifi, 1995; L'taief et al., 2009; Bargaz et al., 2013).

Pea (Pisum sativum L.) is a well-adapted legume to the edapho-climatic conditions of Tunisia. It is a very rich source of protein needed for human nutrition (Ali et al., 2008). Pea is widely grown in various bioclimatic parts of the country. The annual cultivated areas are in the order of 23,000 ha, 70% of this surface is destined for green consumption and 30% is dedicated to the production of dry seeds (DGPA. Ministry of Agriculture, 2014).

The production of pea is, however, considered low, which can be explained by several abiotic factors such as phosphorus deficiency and water stress. This low production is one of the major problems impeding the development of this crop in Tunisia.

The objective of our investigation is to study the effects of inoculation by symbiotic bacteria supposed phosphorus solubilizer, phosphate fertilization and rain regime on the production of biomass, the phosphorus content and phosphorus use efficiency of peas in two different regions of Tunisia in order to recommend more ecological solutions for farmers.

2. MATERIAL AND METHOD

2.1 In vitro Assay of phosphorus solubilization on solid and liquid Pykovskaya (PVK) medium and solubilization index

The two selected bacterial strains of the genus Rhizobium (TAC and 12362) are placed in vitro in Petri dishes containing a tricalcium solid phosphate medium $Ca_3(PO_4)$, prepared according to the method of Pikovskaya (1948). Medium cultures were incubated in an oven at 28°C. The observation under magnification revealed the presence of halo light around the bacterial colonies after 7 days of incubation.

The liquid phosphatic medium is similar to the PVK medium except that it is without agar. The rhizobial colonies are transferred to Erlenmeyer flasks containing the phosphate medium, which are then placed in the oven on a shaker at 150 rpm and a temperature of 28 °C for incubation.

The solubilization index (SI) of the inorganic phosphorus is calculated according to Berraquero et al., (1976) as follows: SI = Halo diameter (mm)/colony diameter (mm). SI <2.00 (weak solubilization); 2.00 ≤ SI <4.00 (medium solubilization) and SI ≥ 4.00 (high solubilization). The optical density (OD) is performed on a spectrophotometer at 630 nm. The optical density and pH measurements are performed for 72 hours.

2.2 Site and experimental design

The trials were conducted during three crop years (2012-2013, 2013-2014 and 2014-2015), located in two different regions of northern Tunisia and belonging to two stations of the National Institute of Agricultural Research of Tunisia (INRAT). The first is at Oued Beja, Beja governorate (Latitude 36, 44° N, Longitude 9.11° E), the second is located at Kef (Latitude 36.10° N, Longitude 8.42° E). The bioclimatic stage of Beja is the lower sub-humid while Kef station is of the semi-arid type to cold winter. These surveys are characterized by irregular and contrasting annual rainfall during the three successive cropping seasons (Figure 1). The rainy season extends from September to June. The average temperature is of the order of 19° C and the soil is of clay texture (Table 1).

Table 1. Physicochemical characteristics of the soil in the superficial horizon (0-20 cm) in the two sites studied

Measured parameters	Oued Béja	Kef
Clay (%)	60	26
Organic matter (%)	2.5	1.41
pH	7.5	7.91
Total nitrogen (%)	2.77	1.9
Phosphorus (mg/kg)	52	16.53

The pea variety used is Lincoln because of its adaptation to the edapho-climatic conditions of Tunisia. Pea crop was carried out in a field of each of both stations with a seeding density of 60 seeds per m². Trials were conducted according to the random block device with three repetitions and six treatments. Each treatment is represented by an elementary parcel with an area of 8 m² (4 m x 2 m) consisting of 4 lines with a spacing of 0.5 m. The first treatment (control) consists of un-inoculated pea plants where phosphorus was not applied. The second consists of a superphosphate supply (45%) at the rate of 200 kg per hectare of P_2O_5.

The third and fourth treatments consist of an inoculation with Rhizobium sp., and with the native strain (S1) TAC of Tunisian origin and the reference strain (S2) 12362 of French origin, chosen from the rhizoteca of the Science Laboratory and Agronomic Techniques of INRAT, for their performances in solubilization of phosphorus. The last two treatments (5^{th} and 6^{th}) include plants inoculated and fertilized with phosphorus. Seed inoculation is carried out by sowing at a rate of 100 ml per elementary parcel. The liquid inoculums used contains more than 109 bacteria per gram. Weeding was done manually. In order to avoid any contamination, the control and phosphate elementary plots were sowed before the inoculated plots. At the flowering stage, sampling was done manually with 12 plants per treatment (with 4 plants harvested per repetition for each block). These plants were then separated into aerial parts, roots and nodules. The roots were washed thoroughly with water and wiped with filter paper.

The nodules are detached from their roots; the dry matter of different plant organs were weighed after drying in an oven at a temperature of 60° C for 72h. The phosphorus dosage was determined by colorimeter using 5 g of finely ground dry matter from the aerial and root part of the plant.

2.3. Data analysis

The data were analyzed statistically by averaging and calculating standard errors. ANOVA was performed for each measured variable. The means were compared using Duncan's test on the basis of least significant differences at 0.05 probability level ($P<0.05$) to test differences between treatments. The data were subjected to a multi-variate analysis using the General Linear Model (GLM) method, including three fixed factors (site, year and treatment) in order to evaluate the effect of each factor and their interaction. The software package used for all these statistical analyze is SPSS (version 20.0; SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Solubilization of phosphorus

The results obtained show that the diameters of the halos formed by two rhizobia strains (S1 and S2) vary from 2.17 to 3.02 mm (Table 2). The optical density and the pH vary on the PVK liquid medium from 0.05 to 0.47 and from 5.7 to 6.4, respectively (Figure. 2). This means that strains S1 and S2 are more acidic to the medium than the control. Moreover, the calculation of the solubilization index (SI) of inorganic phosphorus has revealed that strain S2 (12362) has a solubilization potential of inorganic phosphorus on a $Ca_3(PO_4)^2$ higher than PVK solid medium of the order 6.05 compared to that of strain S2 (TAC) (Table 2).

3.2. Growth of aboveground and root biomass

The results obtained at the Oued Beja station showed significant differences compared with that of Kef station under the different recorded regimes (Figure 3 and Figure 4). A significant increase in the biomass of the aerial and root parts was recorded in response to the addition of phosphate fertilizer added to the inoculation by the bacterial strains TAC (S1)

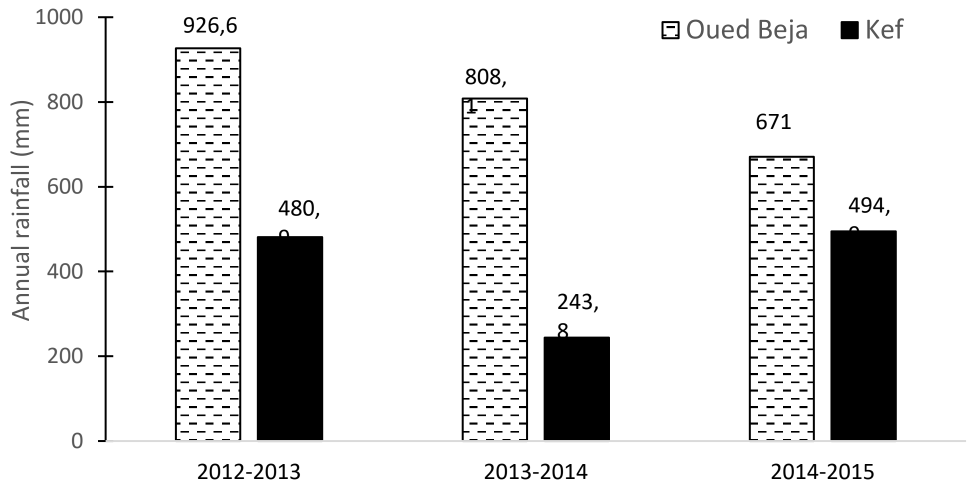


Figure 1. Annual rainfall distribution for the two sites studied (Oued Beja and Kef).

Table 2. Halo diameter and solubilization index of phosphorus on PVK-based medium Ca_3HPO_4 for the two bacterial strains TAC (S1) and 12362 (S2).

Strains	Halo diameter (mm)	Phosphorus solubilization index
S1	2.17± 0.17	4.35±0.33
S2	3.02±0.08	6.05±0.17

The values presented are the means ± Standard error (n = 4) according to the Duncan test ($P<0.05$).

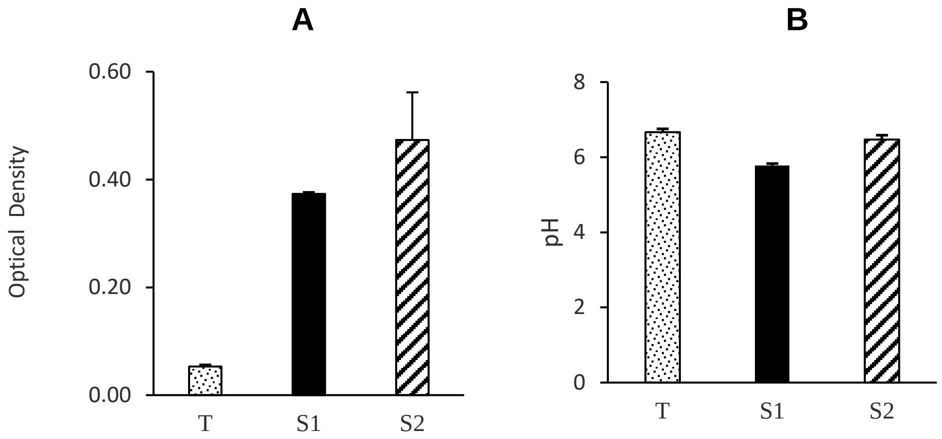
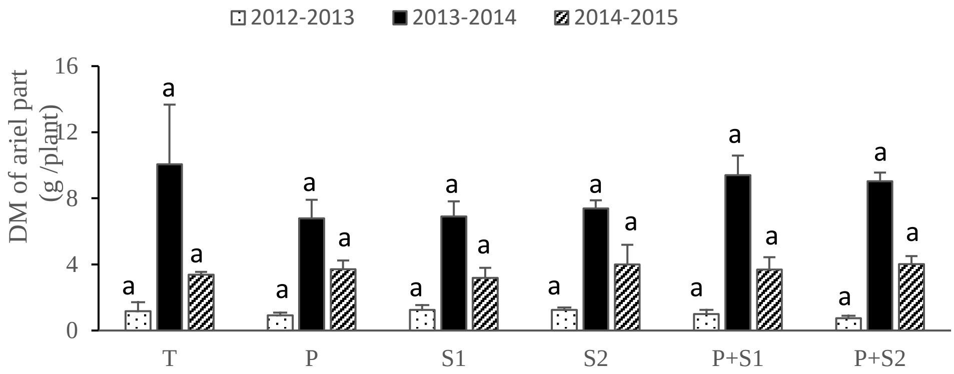


Figure 2. Variation in optical density (A) and pH (B) after incubation on the PVK liquid medium according to the Rhizobium strain tested. (T: control, S1: strain TAC, S2: strain 12362).

A: Oued Beja



B: Kef

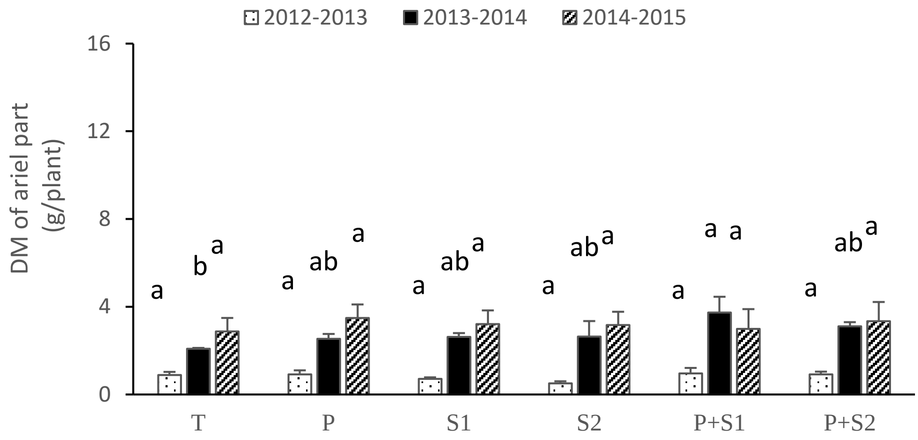
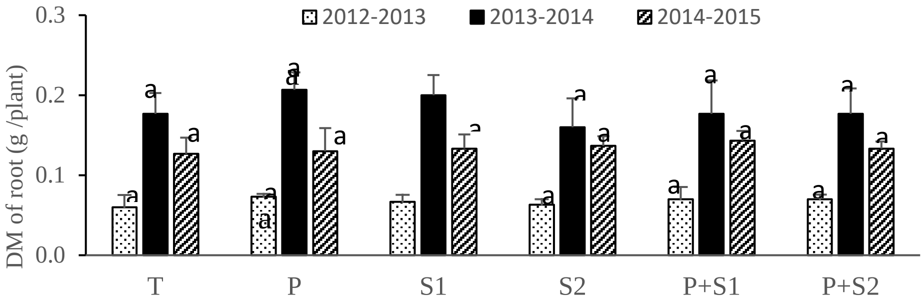


Figure 3. Effect of phosphorus fertilization and inoculation with Rhizobium on dry matter production of aerial part (g $DM.Pl^{-1}$) at flowering stage for Lincoln pea variety. Results of three crop seasons (2012-2013, 2013-2014 and 2014-2015) conducted in two experimental sites of INRAT characterized by contrasted precipitation regimes, (A) Oued Beja and (B) Kef. T: control treatment; P: supply of 200kg phosphorus per hectare. S1: treatment inoculated with TAC strain; S2 treatment inoculated with 12362 strains; P+S1: phosphorus supply combined with inoculated TAC strain; P+S2: phosphorus supply combined with inoculated 12362 strains. Data are the means ± Standard Error of 3 replicates per treatment.

and 12362 (S2) during the three studied campaigns (Figures 3, 4). It appears that a marked effect of phosphorus on pea growth was expressed by a better production in the biomass of the aerial and root parts and which is significantly affected ($P<0.01$) by the site effect and the annual rainfall (Table 3).

A: Oued Beja



B: Kef

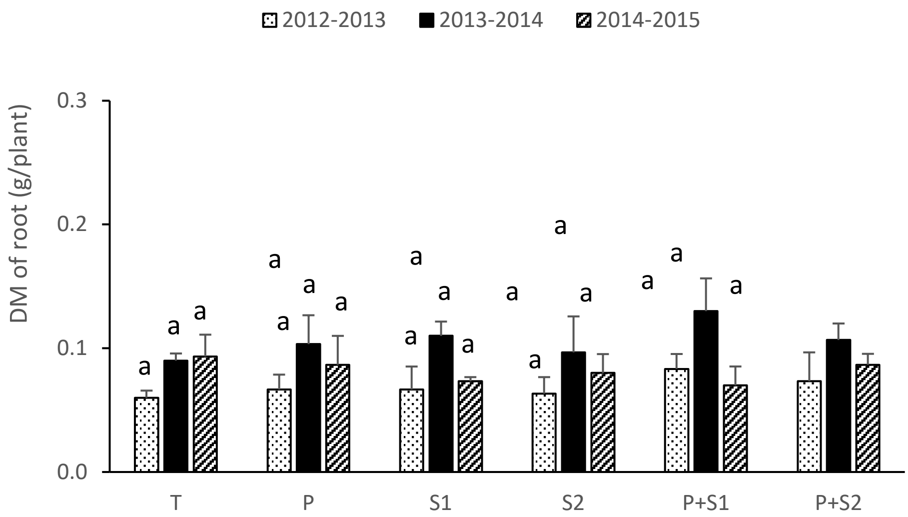


Figure 4. Effect of phosphorus fertilization and inoculation with Rhizobium on dry matter production of roots (g DM.Pl^{-1}) at flowering stage for Lincoln pea variety. Results of three crop seasons (2012-2013, 2013-2014 and 2014-2015) conducted in two experimental sites of INRAT characterized by contrasted precipitation regimes, (A) Oued Beja and (B) Kef. T: control treatment; P: supply of 200kg phosphorus per hectare. S1: treatment inoculated with TAC strain; S2: treatment inoculated with 12362 strains; P+S1: supply of phosphorus combined with inoculated TAC strain; P+S2: supply of phosphorus combined with inoculated 12362 strains. Data are the means ± Standard Error of 3 replicates per treatment.

3.3. Growth of nodular biomass

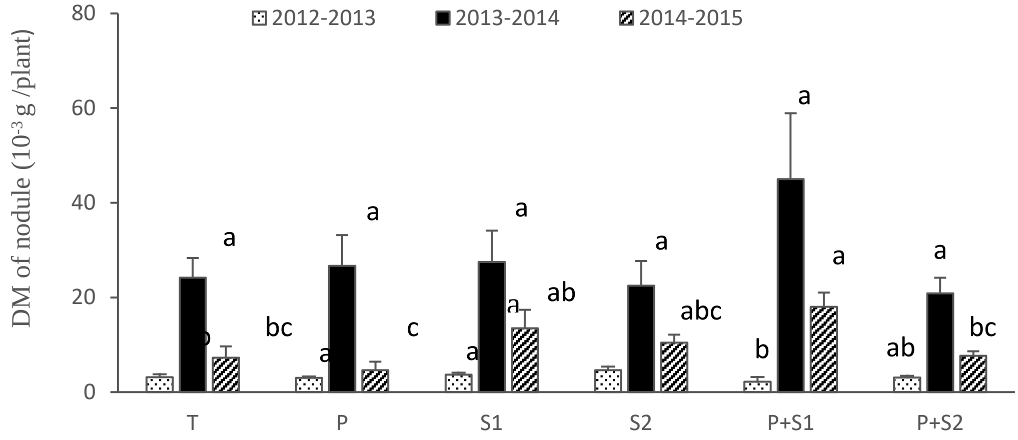
The variations of the nodular biomass as a function of the treatments recorded during the three campaigns in the two stations (Oued Beja and Kef) are illustrated in figure 5. In the two studied sites, an inter-annual and highly significant variation of nodular biomass was noted for phosphorus and inoculated treatments compared to that of the control (Figure 5). Inoculation treatments with Rhizobium strains combined with phosphorus intake have the highest nodule biomass at the Kef site compared to Oued Beja (Figure 5). Nodular growth appears to be dependent on moisture conditions and is greater under the rainfall regime recorded during the two years 2013-2014 and 2014-2015. However, this effect is weak in the Oued Beja station (Figure 5). Inoculated treatments (S1 and S2) induced an improvement in nodular growth in the Kef station compared to that of Oued Beja (Figure 5). It was found that nodulation is significantly affected ($P<0.01$) by site effect and annual rainfall (Table 3).

3.4. P content and phosphorus use efficiency (PUE)

The phosphorus content and its distribution in the aerial and root parts of peas vary considerably according to the annual rainfall and experimental station (Tables 4 and 5). Regardless of site, the obtained results showed that the amount of phosphorus in the aerial part is greater relative to that of the roots (Tables 4 and 5). As well as, it shows a great spatial variability of the plot cultivated in peas in the Oued Beja station compared to that of the Kef station (Tables 4 and 5). Thus, it appears that the phosphorus content in the aerial and root parts is significantly affected ($P<0.01$) by the site and the annual rainfall regime effects (Table 3).

The effect of P supply and inoculation with rhizobia on absorption and utilization efficiency of phosphorus in relation to dry matter production and nitrogen fixation were examined in Lincoln variety pea with different treatments during flowering stage under open field conditions (Table 6). Phosphorus use efficiency (PUE= Arial biomass / Phosphorus content) is more important in Oued Beja station comparing to Kef station during the both lasted crop seasons (2013-2014 and 2014-2015). The results showed that at Oued Beja station PUE is slightly improved by phosphorus supply and the inoculation by the both of rhizobia (S1: TAC and S2:12362). While at Kef station, the PUE was lower and significantly affected by the climatic conditions.

A: Oued Beja



B: Kef

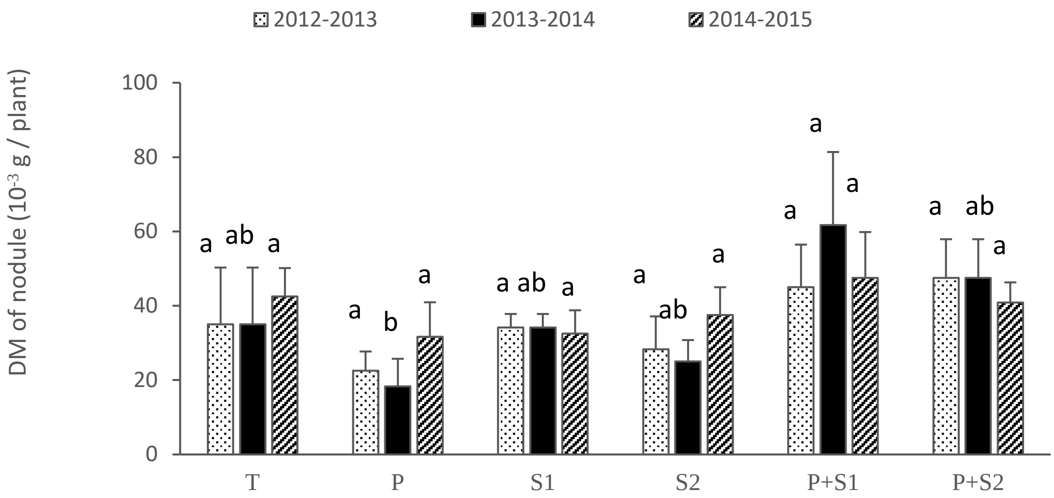


Figure 5. Effect of phosphorus fertilization and inoculation with Rhizobium on node growth (g DM Pl^{-1}) at flowering stage for Lincoln pea variety. Results of three crop seasons (2012-2013, 2013-2014 and 2014-2015) conducted in two experimental sites of INRAT characterized by contrasted precipitation regimes, (A) Oued Beja and (B) Kef. T: control treatment; P: supply of 200kg phosphorus per hectare. S1: treatment inoculated with TAC strain; S2: treatment inoculated with 12362 strains; P+S1: supply of phosphorus combined with inoculated TAC strain; P+S2: supply of phosphorus combined with inoculated 12362 strains. Data are the means ± Standard Error of 3 replicates per treatment.

Table 3. Effects of different factors (site, year and treatment) on the parameters studied.

Parameters	Site effect, *F calculated*	Year effect, *F calculated*	Treatment effect, *F calculated*	Site × year, *F calculated*
Aerial biomass	55.57**	91.76**	0.60 *NS*	37.97**
Root biomass	42.73**	46.25**	0.41 *NS*	12.72**
Nodular biomass	78.99**	8.34**	4.01**	7.40**
Aerial part Phosphorus content	85.83**	20.18**	0.62 *NS*	20.08**
Roots Phosphorus content	85.92**	8.29**	0.58 *NS*	5.50 **
PUE	342.66**	58.87**	0.25*NS*	66.99**

**: highly significant at P <0.01, NS: not significant.

Table 4. Spatial and temporal variation of the phosphorus content (mg g^{-1} MS) of the aerial part of the Lincoln variety of pea grown in two experimental stations (Oued Beja and Kef) during three cropping seasons (2012-2013, 2013-2014 and 2014-2015).

Sites	treatment	2012-2013	2013-2014	2014-2015
Oued Béja	control	15.4± 2.70a	24.74±7.22a	8.42±0.93a
	P	9.25±0.98a	14.11±1.83a	8.63±1.76a
	S1	15.67±1.20a	17.60±3.65a	7.62±0.83a
	S2	11.70±1.73a	15.78±1.80a	8.78±3.10a
	P+S1	11.20±1.65a	17.37±1.59a	8.01±1.37a
	P+S2	11.36±1.58a	20.03±4.90a	9.69± 0.10a
	F calculated	1.4 *NS*	0.84 *NS*	0.17 *NS*
Kef	control	1.30 ± 0.46a	3.89±0.71c	5.56±2.13a
	P	2.32± 0.70a	7.41±1.21abc	10.28±3.16a
	S1	1.11± 0.23a	5.31±0.16bc	6.16± 0.96a
	S2	1.12±0.32a	7.30±2.70bc	8.18±2.18a
	P+S1	2.55±0.69a	11.72±2.02a	10.29±4.80a
	P+S2	2.33±0.49a	9.35±1.28ab	9.26±1.66a
	F calculated	1.73 *NS*	3.11*	0.54 *NS*

*Values are means ± Standard Error (n=3) according to Duncan's multiple range test (P<0.05). *Significant at P < 0.05; NS: not significant.*

4. DISCUSSION

The use of phosphate solubilizing bacteria as bacterial inoculants plays an important role in plant nutrition through the increase in phosphorus uptake by the plant. In the present study, the variability of the diameters of the halos formed by two Rhizobium strains (S1 and S2) is an indicator of the ability of strains to solubilize inorganic phosphorus. As, the solubilization index (SI) of inorganic phosphorus (Pi) confirms the ability of two selected strains (S1 and S2) nodulating pea to solubilize inorganic phosphorus (Berraquero et al., 1976).

Optic density results suggest that these bacteria are able to produce organic acids during their growth. In this context, Yi et al., (2008) indicates that the solubilization of different sources of phosphorus by gram-negative bacteria is mainly due to the production of organic acids. Lin et al. (2006) demonstrated that pH decrease is a critical factor in phosphate solubilization that is dependent on the production of organic acid and protons. Similar results have been observed by Chung et al., (2005) who showed that the high solubilization of tri-calcium phosphate $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$ on PVK liquid medium was observed with bacterial strains extracted from the rhizosphere of different species such as green onion (Allium fistulosum L.), chilli pepper (Capsicum annuum L.), sesame (Sesamum indicum L.) and rice (Oryza sativa L.). Similarly, Marra et al., (2011) showed that most of the bacterial strains of the genus Bradyrhizobium nodulating cowpea and Rhizobium tropici CIAT 899T nodulating the bean have a high potential for inorganic phosphorus solubilization especially on the solid medium PVK based on tri-calcium phosphorus $Ca_3(PO_4)_2$.

Table 5. Spatial and temporal variation of the phosphorus content (mg.g^{-1} DM) of the root part of the Lincoln variety of pea grown in two experimental stations (Oued Béja and Kef) during three cropping seasons (2012-2013, 2013-2014 and 2014-2015).

Sites	Treatment	2012-2013	2013-2014	2014-2015
Oued Béja	control	0.62±0.11a	0.54±0.06a	0.40±0.07a
	P	0.43±0.04a	0.65±0.11a	0,40±0.11a
	S1	0.56±0.09a	0.64±0.05a	0.40±0.06a
	S2	0.53±0.06a	0.46±0.13a	0.39±0.06a
	P+S1	0.43±0.04a	0.49±0.06a	0.47±0.05a
	P+S2	0.53±0.07a	0.50±0.06a	0.45±0.07a
	F calculated	0.95 *NS*	0.61 *NS*	0.18 *NS*
Kef	control	0.12±0.02bc	0.23±0.03a	0.23±0.06a
	P	0.18±0.03abc	0.32±0.06a	0.26±0.04a
	S1	0.05±0.05c	0.38±0.11a	0.28±0.08a
	S2	0.15±0.02abc	0.30±0.11a	0.23±0.03a
	P+S1	0.27±0.04a	0.47±0.05a	0.26±0.06a
	P+S2	0.23±0.06ab	0.41±0.07a	0.31±0.03a
	F calculated	3.27*	1.05 *NS*	0.31 *NS*

*Values are means ± Standard Error (n=3) according to Duncan's multiple range test (P<0.05). *Significant at P < 0.05; NS: not significant.*

Growth of aboveground and root biomass results suggest that the phosphate fertilizer could be substituted by biological fixation via the two strains of Rhizobium. Thus, Inoculation with both selected strains plays the role of PGPR (Plant growth Promoting Rhizobacteria) and their use as biofertilizers. Furthermore, Chabot et al, (1996 ab) concluded that inoculation with two strains of Rhizobium leguminosarum selected for their phosphate solubilization ability has been shown to improve root colonization and growth promote and to increase significantly the phosphorus content in maize and lettuce. Similar studies of pea (Akhtar et al., 2003), beans (Abdi et al., 2012, Zaman-Allah et al., 2007) have shown that vegetative growth is dependent on rhizobia inoculation, the variety and application of phosphate fertilization. As, several soil microorganisms, including bacteria, improve the supply of phosphorus to plant as a consequence of their capability for inorganic phosphate solubilization (Richardson, 1994). Similarly, other studies on the beans inoculated with the Rhizobium Tropici strain (CIAT899), have shown that the application of phosphorus increases the leaf area, the total biomass of the dry matter, nodular and root biomasses of the plant (Olivera et al., 2004, Kouki et al., 2016). In addition, phosphate treatment combined with inoculation with rhizobia nodulating bean further contributed to improved biomass growth in aerial and root parts compared to the control (Abdi et al., 2014; Bargaz et al., 2012). In addition, recent studies have reported that phosphorus may play a key role in the transfer of energy required for growth and improved plant productivity (Ribet & Drevon 1996, Hmissi et al., 2015).

Growth of nodular biomass results corroborate with those obtained by Kouki et al., (2016) and Hmissi et al., (2015) who showed that inoculation with Rhizobium strains contributes significantly to the improvement of nodular biomass and the increase in the number of nodules. Other previous studies of legume symbiosis-rhizobia have addressed the requirement for nodulation and nodular growth for phosphorus (Drevon & Hartwig 1997, Ribet & Drevon 1996, Abdi et al., 2014). This could be explained by the strong positive correlation between phosphorus availability and nodulation involving a series of interactions between the host plant and the bacterium (Lynch et al., 1991). Kaouas et al., (2005) also revealed that phosphate nutrition affects nodule formation and the functioning of symbiosis.

Phosphorus content indicates that phosphorus mobility from the roots to the aerial part is rapid. On the other hand, a better solubilization of the inorganic phosphorus of the soil is ensured by the strains of Rhizobium S1 and S2. This is confirmed by the previously calculated phosphorus solubilization index that is of the order of 4 and 6 for S1 and S2, respectively. Indeed, absorption and assimilation of phosphorus are dependent on rainfed conditions. Our results suggest that P plays an important role in symbiotic fixation through an effective translocation of P to the leaf. Thus, when P supply was applied alone or combined with rhizobium strain to pea plant, dry matter production reasonably high through an effective translocation of the absorbed P to the leaf. Dry matter production and nitrogen symbiotic fixation are strongly controlled by P absorption ability rather than phosphorus utilization efficiency (PUE). At Kef station, the low absorption ability of phosphorus during short flowering stage is mainly due to poor root development. Our findings showed that P increases symbiotic nitrogen fixation by stimulating plant growth rather than exerting a direct effect on nodule initiation, growth and development (Adu-Gyamfi et al, 1989; Sandana & Pinochet, 2014).

In this context, studies of legumes grown on a regional scale using climate and environmental data have shown a strong correlation between soil moisture status and nutrient availability (Sifi, 1995; Akhtar et al., 2003, L'taief et al., 2009). Our study also confirms the results of several studies that have shown the importance of microbial activity in the bioavailability of inorganic phosphorus to plants (Abdi et al., 2012; Hmissi et al., 2015; Kouki et al., 2016). In the same time, a higher P solubility may facilitate leaching of fertilizers, in this case phosphorus, from permeable soils during rainy seasons, as some scientists recently showed (Lacatusu et al., 2019, Paltineanu et al., 2021)

5. CONCLUSION

This Study showed that the two rhizobia strains isolated from pea roots had a high solubilization capacity of phosphorus with a solubilization index greater than four. The judicious choice of the strains selected (TAC: S1) and (12362: S2) allowed an increase in aerial, root and nodular biomass as well as in the number of nodules for both fertilization and inoculation. The increase in the number of nodules is generally limited by several factors that are cited as the presence of native strains highly competitive for the nodulation of pea as is the case of the TAC strain which has a better inoculation potential than the 12362 strain.

Variation in total pea biomass and solubilization of phosphorus are mainly influenced by rainfall. Between the three stations studied, the tested modalities of production factors (phosphate fertilization and bacterial inoculation) led to a significant spatial variability in the dry matter yield potential of the various pea organs (aerial part, roots and nodules). In addition, inter-annual variations in yield were greater than the observed effects of the factors tested. Despite, the seemingly limited effects of inoculation, it is acceptable to conclude in the first analysis that inoculation of peas with Rhizobium strains (S1 and S2) could substitute phosphate fertilizer to improve pea production, the phosphorus content absorbed by the plant and particularly root development.

Moreover, it is essential to confirm these early trends as well as it would be necessary to study deeply the mechanism of solubilization of phosphate and to well understand the interactions existing on the solubilization in the rhizosphere, in particular with the edaphic factors (pH and limestone contents). In addition, it would be indispensable to study, their effects on the bioavailability of phosphorus and other nutrients necessary for the nutrition of the plant. Further, studies should be undertaken to compare the symbiotic performance of Rhizobia strains nodulating pea (under phosphorus deficiency) with regards to the climatic conditions of the region to promote pea cultivation and increase symbiotic nitrogen fixation as well as grain yields.

Acknowledgements

This work was supported by the spin division of the National School of Mines of Saint Etienne, the National Institute for Agricultural Research in Tunisia, the National Agronomic Institute of Tunisia and the National Institute for Research in Rural Engineering, 'Water and forestry. The authors thank the anonymous reviewer who allowed us to improve the drafting of this article.

REFERENCES

Abdi N., Bargaz A., Faghire M., Bouraoui M., Hmissi I., L'taief B., Ghoulam C. & Sifi B., 2012. *Phaseolus vulgaris-rhizobia symbiosis increases the phosphorus uptake and symbiotic N2 fixation under insoluble phosphorus*. Journal of plant Nutrition, 37, 643-657.

Abdi N., L'taief B., Hemissi I., Bouraoui M., Maazaoui H. & Sifi B., 2014. *Nitrogen and phosphorus fertilization effect on Rhizobia-common bean symbiosis.* Annales de l'INRAT, 87, 21-33.

Adu-Gyamfi J.J., Fujita K. & Ogata S., 1989. *Phosphorus absorption and utilization efficiency of pigeon pea (Cajanus cajan (L) Mill sp.) in relation to dry matter production and dinitrogen fixation.* Soil and Plant, 119, Issue 2, 315–324

Akhtar N., Amjad M. & Anjum M.A., 2003. *Growth and yield response of pea (Pisum sativum L.) crop to phosphorus and potassium application.* Pakistan Journal of Agricultural Sciences, 40, 217-222.

Ali M.E., Khanam D., Bhuiyan M.A.H., Khatun M.R. & Talukder M.R., 2008. *Effect of Rhizobiuminoculation to different varieties of gardenpea (Pisum sativumL.).* J. Soil. Nature, 2, 30-33.

Attar H.A., Blavet D., Selim E.M., Abdelhamid M.T. & Drevon J.J., 2012. *Relationship between phosphorus status and nitrogen fixation by common beans (Phaseolus vulgaris L.) under drip irrigation.* International Journal of Environmental Sciences and Technology, 9, 1-13.

Bargaz A., Faghire M., Abdi N., Farissi M., Sifi B., Drevon J.J., Ikbal M.C. & Ghoulam C., 2012. *Low soil phosphorus availability increases acid phosphatases activities and affects P partitioning in nodules, seeds and rhizosphere of Phaseolus vulgaris.* Agriculture, 2, 139-153.

Bargaz A., Faghire M., Farissi M., Drevon J.J. & Ghoulam C., 2013. *Oxidative stress in the root nodules of Phaseolus vulgarisis induced under conditions of phosphorus deficiency.* Acta physiologia Plantarium, 35, 1633-1644.

Benadis C., Bekki A., Khoulassa S. & Drevon J.J., 2014. *Mycorrhizal colonization decreases respiration of common bean nodulated root in hydroaeroponic culture*. Simbiosis, 62, 135-142.

Berraquero F.R., Baya A.M. & Cormenzana A.R., 1976. *Establishment of indices for the study of phosphate solubilization by soil bacteria.* Ars Pharmacéutica, 17, 399-406.

Chabot R, Antoun H, Kloepper JW, & Beauchamp CJ., 1996a. *Root colonization of maize and lettuce by bioluminescent Rhizobium leguminosarum biovar. phaseoli.* App Environ Microbiol; 62: 27, 67-72.

Chabot R, Hani A. & Cescas PM. 1996b. *Growth promotion of maize and lettuce by phosphate-solubilizing Rhizobium leguminosarum biovar. phaseoli.* Plant Soil; 184: 311-21.

Chung H., Park M., Madhaiyan M., Seshadri S., Song J., Cho H. & Sa T., 2005. *Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea.* Soil Biology and Biochemistry, 37, 1970-1974.

Drevon J.J. & Hartwig U.A., 1997. *Phosphorus deficiency increases the argon-induced decline of nodule nitrogenase activity in soybean and alfalfa.* Planta, 201, 463-469.

Hinsinger P., 2001. *Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review.* Plant and Soil, 237, 173-195.

Hmissi I., Abdi N., Bargaz A., Bouraoui M., Mabrouk Y., Saidi M. & Sifi B., 2015. *Inoculation with phosphate solubilizing Mezorhizobium strains improves the performance of chickpea (Ciceraritenium L.) under phosphorus deficiency.* Journal of Plant Nutrition, 38, 1656-1671.

Kaouas S., Labidi N., Debez A. & Abdelly C., 2005. *Effect of P on nodule formation and N fixation in bean.* Agronomy for sustainable Development, 25, 389-393**.**

Kouki S., Abdi N., Hemissi I., Bouraoui M. & Sifi B., 2016. *Phosphorus fertilization effect on common bean (Phaseolus vulgaris L.)-rhizobia symbiosis.* Journal of New Sciences- Agriculture and Biotechnology, 25, 1130-1137.

Lacatusu R., Paltineanu C., Vrinceanu A. & Lacatusu A.R. 2019. *Influence of domestic activity on the quality of groundwater and surface water in the rural built-up area of the southern Romanian Danube Plain – a case study in the Glavacioc catchment.* Carpathian Journal of Earth and Environmental Sciences, Volum 14(2): 323-334. Doi:10.26471/cjees/2019/014/083.

Latati M., Balvet D., Alkama N., Laoufi H., Drevon J.J., Gérard F., Pansu M. & Ounane S.M., 2014. *The intercropping cowpea-maize improves soil phosphorus availability and maize yields in an alkaline soil.* Plant and Soil, 385, 181-191.

Lin T.F., Huang H.I., Shen F.T. & Young C.C., 2006. *The protons of gluconic acid are the major factor responsible for the dissolution of tri-calcium phosphate by Burkholderia cepacia CC-Al74.* Bioresource Technology, 97, 957-960.

L'taief B., Sifi B., Zaman-Allah M., Hajji M. & Lachaal M., 2009. *Effets de la fertilisation azotée, de l'inoculation par Rhizobium sp. et du régime des pluies sur la production de la biomasse et la teneur en azote du pois chiche. Biotechnol.* Agron. Soc. Environ., 13, 537-544.

L'taief B., Sifi B., Zaman-Allah M., Horres R., Molina C., Beebe S., Winter P., Kahl G., Drevon J.J. & Lachaal M., 2011. *Genotypic variability for tolerance to salinity and phosphorus deficiency among N2-dependent recombinant inbred lines of common bean (Phasoelus vulgaris).* African Journal of Microbiology Research, 6, 4205-4213.

Lynch J., Lauchli A. & Epstein E., 1991. *Vegetative growth of the common bean in response to phosphorus nutrition.* Crop Science, 31, 380-387.

Marra L.M., Maria de Oliveira S., Fonsêca Sousa Soares C.R. & Moreira F.M.S., 2011. *Solubilization of inorganic phosphates by inoculant strains from tropical legumes*. Scientia Agricola, 68, 603-609.

Marschner P., Crowley D. & Rengel Z., 2011. *Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis-model and research methods.* Soil Biology and Biochemistry, 43, 883-894.

Ministère de l'Agriculture, 2014. *Budget économique 2015, Agriculture et Pêche. Ministère de l'Agriculture et -des Ressources Hydrauliques*, Tunisie, Novembre-Décembre 2015.

Oehl F., Oberson A., Probst M., Fliessbash A., Roth H.R. & Frossard E., 2001. *Kinetics of microbial phosphorus uptake in cultivated soils.* Biology and fertility of soils, 34, 31-41.

Olivera M., Tejera N., Iribarne C., Ocaña A. & Lluch C., *Growth, nitrogen fixation and ammonium assimilation in common bean (Phaseolus vulgaris): effect of phosphorus.* Physiologia Plantarum, 121, 498-505.

Paltineanu C., Domnariu H., Marica D., Lacatusu A.R., Popa G.A., Grafu I.A. & Neagoe A.D. 2021. *Fertilizers` leaching from the root system zone – a potential environmental risk for groundwater pollution in coarse and medium-textured soils.* Carpathian Journal of Earth and Environmental Sciences, 16(1): 139–150. Doi:10.26471/cjees/2021/016/162.

Park J.H., Bolan N., Megharaj M. & Naidu R., 2011. *Comparative value of phosphate sources on the immobilization of lead, and leaching of lead and phosphorus in lead contaminated soils.* Science of the Total Environment, 409, 853-860.

Pikovskaya R.I., 1948. *Mobilization of phosphorus in soil in connection with vital activity of some microbial species.* Mikrobiologya, 17, 362-370.

Ribet J. & Drevon J.J., 1996. *The phosphorus requirement of N2 fixation and urea-fed Acacia mangium.* New Phytol., 132, 383-396.

Richardson A.E., 1994. *Soil microorganisms and phosphorus availability.* In: Pankhurst CE, Doube BM, Grupta VVSR, Grace PR, editor. Sol Biota, Management in sustainable Farming Systems. Melbourne, Australia: CSIRO, pp, 50-62.

Richardson A.E., 2001. *Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants.* Australian Journal of plant physiology, 28, 897-906.

Rosas S.B., Andrés A.J., Rovera M. & Correa N.S., 2006. *Phosphate-solubilizing Pseudomonas putida can influence the rhizobia–legume symbiosis.* Soil Biology and Biochemistry, 38, 3502-3505.

Sandana P. & Pinochet D., 2014. *Grain yield and phosphorus use effeciency of wheat and pea in high yielding environement.* Journal of Soil Science and Plant Nutrition, 14 (4), 973-986.

Schulze J., Mohamed M.A.N., Carlsson G. & Drevon J.J., 2011. *Phosphorous deficiency decreases nitrogenase activity but increases proton efflux in N2-fixing Medicago truncatula.* Plant Physiology and Biochemistery, 49, 458-460.

Sifi B., 1995. *Etude de la survie de Rhizobium tropici sur différents substrats et leur influence sur la cultue du haricot.* In J.J. Drevon (ed.): Facteurs limitants la fixation symbiotique de l'azote dans le bassin méditerranéen. INRA Paris, Les Colloques 77, 277-284.

Sundara B., Natarajan V. & Hari K., 2002. *Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields.* Field Crops Research, 77, 43-49.

Tajini F. & Drevon J.J., 2014. *Phosphorus use efficiency for symbiotic nitrogen fixation varies among common bean recombinant inbred lines under P deficiency*. Journal of Plant Nutrition, 37, 532-545.

Yi Y., Huang W. & Ge H., 2008. *Exopolysaccharide: a novel important factor in the microbial dissolution of tri-calcium phosphate.* World Journal of Microbiology and Biotechnology, 24, 1059-1065.

Zaman-Allah M., Sifi B., L'taief B., El Aouni M.H. & Drevon J.J., 2006. *Rhizobial inoculation and P fertilization response in common bean (Phoseolus vulgaris L.) under glass house and field conditions.* Experimental Agriculture, 43, 1-10.

Zaman-Allah M., Sifi B., L'taief B., El Aouni M.H. & Drevon J.J., 2007. *Symbiotic response to low phosphorus supply in two common bean (Phaseolus vulgaris) genotypes*. Symbiosis, 43, 109-113.

Received at: 09. 06. 2022
Revised at: 25. 06. 2022
Accepted for publication at: 30. 06. 2022
Published online at: 09. 07. 2022