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Phosphorus fertilization effect on common bean (*Phaseolus vulgaris* L.)-rhizobia symbiosis

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Abstract - Response to mineral fertilization, especially phosphorus (P), and the lack of efficient rhizobia strains in tunisian soil, where P deficiency, is one of the major factor limiting symbiotic nitrogen fixation (SNF) and yield of *Phaseolus vulgaris* L. In order to select the efficient strain and to study how P fertilization may improve rhizobial inoculation and there by symbiosis yields, greenhouse experiment and field trials in two different bioclimatic regions of Tunisia (Oued Beja and Oued Méliz) were conducted. Under greenhouse conditions, using Coco Blanc that is characterized a more rentable variety, fifty four strains have been tested. In field conditions, six treatments were completed: (T: Control), (P: 200Kg/ha), (CIAT899), (Ar02), (CIAT899+P: CIAT899+200Kg/ha), (Ar02+P: Ar02+200Kg/ha). Results showed that nodulation evaluation revealed variability among the 54 rhizobia strains. In compared with controlled and other strains, Ar02 showed higher increase nodulation number in order to 134 nodules per plant. Field trials showed that inoculation and P supply increased mainly in Oued Beja nodulation (24 nodules per plant), N content (2.1%) and shoot dry weight (2.44g $plant^{-1}$). In comparison with other combinations, phosphorus fertilizer supply and rhizobia inoculation ameliorated mainly in Oued Beja, the nodulation(16.9 nodules per plant) and nodule dry weight (0.27g $plant^{-1}$) under field conditions.

Keywords: biomass; common bean; nodulation; nitrogen; rhizobia; yield.

1. Introduction

Legumes are the most important source of proteins for direct human consumption with common bean (*Phaseolus vulgaris* L.) comprising 50% of the grain legumes consumed worldwide (Bargaz et al. 2012; Abdi et al. 2014). These leguminous crops are commonly considered efficient restorative agents for soil fertility. The symbiotic association between common bean roots and rhizobia leads to formation of root nodules, where symbiotic nitrogen fixation (SNF) takes place. Estimates for field grown legumes revealed that up to 80% of the plant nitrogen demand is met by N2 fixation in these species (Larue and Patterson 1987). However, several environmental factors are important constraints worldwide for leguminous crops and particularly for common bean production in most farms where this crop is grown (Zaman Allah et al. 2006). The soil P deficiency is one of the most significant abiotic factors, along with N, limiting crop productivity. Overall, it is reported that 40% of crop production in the world's arable land is limited by P availability and sub-optimal levels of P can result in 5 to 15% yield losses (Bargaz et al. 2012). The symbiotic process between legume roots and bacteria, phosphate has received considerable attention due to the dramatic effects observed in low-phosphate soils when P fertilizer is applied to nodulated legumes, including *Phaseolus vulgaris* L. (Zaman Allah et al. 2006; Abdi et al. 2014). The contribution of phosphorus in plants inoculated has a significant effect on the nitrogen content and the increase was more than 48% compared to control plants (Hmissi et al. 2015). In comparison with other legume, (Vadez et al. 1996; Zaman Allah et al. 2006) confirm the high sensitivity of symbiotic nitrogen fixation to the type of fertilization in legume.Under limiting P conditions, legumes may lose the distinct advantage of an unlimited source of symbiotic N2, decreases in N2 fixation leading to decreases in plant growth and nodulation (Vadez et al. 1996). However, the mechanism of P limitation's effect on the N2 fixation process is not fully understood (Vance 2001; Hellsten and Huss Danell 2001). Under limited conditions of P, the optimum symbiotic interaction between the host plant and rhizobia would depend on efficient allocation and use of available P (Vance 2001). P level optimal was in order to 4.3 mg P kg^{-1} (Bargaz et al. 2012). Improving P nutrition to legumes under P-deficient conditions has generally involved two major mechanisms: (i) increasing P acquisition (root morphology, root exudation and P uptake mechanisms); and (ii) enhancing P utilization by internal mechanisms associated with conservable use of absorbed P at the cellular level (Raghothama 1999; Vance 2001; Bargaz et al. 2012). Application of bacterial inoculants and P fertilizer to field increased biomass production and grain yield of common bean compared with the single use of P or rhizobial strains (Abdi et al. 2014). Thus, the aim of this work was, to select the efficient rhizobia- common bean symbiosis. In addition, we studied the effect of P fertilization on rhizobia strain efficiency and it impact on symbiotic nitrogen fixation, nodulation, plant and grain yield under field conditions.

2. Materials and methods

2.1. Nodulation test

The seeds of Coco Blanc variety of common bean were sterilized with calcium hypochlorite (6.7%) for 15 min and then washed carefully in 4 changes of sterile distilled water. Thereafter, seeds were germinated for three days in Petri dishes containing sterile moistened blotting paper. Local and introduced rhizobia and common bean seeds were provided by the Laboratory of Sciences & Techniques Agronomics; National Institute of Agronomic Research in Tunisia (INRAT, Tunisia) (Table 1). Rhizobial inoculants, prepared as a liquid culture in YEM medium (Vincent 1970), were applied by soaking seedlings for 30 minutes in the inoculants prior to transplanting in plastic growth pots (0.5kg of sterile perlite). The ability of 54 infective strains was conducted through the measurement of parameters of the nodulation bearing on the number and nodule biomass. The test consists of 55 treatments. Each treatment was repeated 4 times. Irrigation was performed at 40 ml per pot 2 times a week with a nutrient solution devoid of nitrogen (Vincent 1970). Treatments are shown in Table1.

Table 1. Origin of rhizobia strains used for testing nodulation

N°	Reference	Origin	N°	Reference	Origin
1	CIAT899	International Center for Tropical	28	KHT1.96	Nabeul
2	Alia1	Bizerte	29	KHT3.96	Nabeul
3	Alia2.96	Bizerte	30	Ar3	Ariana
4	Tinja	Bizerte	31	Ar1	Ariana
5	Ar02	Ariana	32	Ar6	Ariana
6	Ar05	Ariana	33	Ar4	Ariana
7	P.Ar.09	Ariana	34	Ar2	Ariana
8	P.Bj	Beja	35	S1	Ariana
9	P.OM.09	Oued Meliz	36	J1.96	Bizerte
10	P.Ps. 09	Phosphate Gafsa	37	J2.96	Bizerte
11	CB	Cap bon	38	J3.96	Bizerte
12	P.Tb.09	Teboursek	39	J1.92	Bizerte
13	SOM	Maroc	40	J3.92	Bizerte
14	D4.007	INRA Montpellier	41	J4.92	Bizerte
15	D4.002	INRA Montpellier	42	S3	Ariana
16	KHS1	INRA Montpellier	43	S7	Ariana
17	KHS2	INRA Montpellier	44	S9	Ariana
18	GB.92	INRA Montpellier	45	S11	Ariana
19	GB.258	INRA Montpellier	46	Raf .Raf	Bizerte
20	KH28	INRA Montpellier	47	Ras.JB	Bizerte
21	Fr1.97	Fernana	48	Soudan1.2	Nabeul
22	OM	Oued Méliz	49	Soudan2.2	Nabeul
23	Mat.9	Mateur	50	D2.2	Bizert
24	Zaar	Mateur	51	D3.2	Bizert
25	ZG.96	Zaghouan	52	Artn1	Ariana
26	B155	CIRAD Montpellier	53	Ic.208	Ariana
27	S10	Ariana	54	12a3	Ariana

2.2. Field trials

The field trials were conducted to assess variety ×strain ×site interactions on nodulation, nitrogen fixation, biomass accumulation and grain yields at late February to early June in northern Tunisia, in two experimental stations of INRAT in Beja (36.44 N, 9.11 E) and Oued Meliz (36.28 N, 8.29 E). In Beja, the annual mean rainfall is 560 mm with a median air temperature of 19°C; the soil is a vertisol with an average content of available P and total N of 32 and 2.77mg Kg^{-1}, respectively. At Oued Meliz, the annual mean rainfall was 462 mm with a median temperature of 19°C; the soil is sandy and clay with an average content of available P and total N of 39 and 2.18 ppm, respectively. Trials were carried out in a complete randomized block ($8m^2$) design with four replicates using the same treatments as in glasshouse. Seeds were sown in late February at a density ranging from 25 to 30 per m^2. Trial in field conditions was done as a confirmation to the study of (Abdi et al. 2014). The use of the highest maximum dose of phosphorus on field common bean culture (90 U.P as 200Kg/ha superphosphate 45%) was in order to confirm results obtained by (Abdi et al. 2014) in the same sites and culture.

2.3. Harvest and data analysis

Under greenhouse conditions, four plants for each treatment were harvested at the early flowering stage. Nodules were then removed from the roots and the plants were separated into shoots and roots and dried in an oven at 70°C for 72 h. After dry weight measurements, shoots of each sample were ground individually and the N content was measured using the Kjeldahl procedure. For the field trials, complete systems with nodules of four plants were collected. Then, after rinsing them carefully, the roots and shoots of each plant were placed in paper bags. For each treatment, a total of 16 plants (four samples per block and four blocks in total) were harvested at flowering stage. Symbiotic parameters (nodule number and nodule dry weight (NDW)), shoots dry weights (SDW), nitrogen content per plant (%N/Pl) at flowering stage and yield at maturity stage were measured on each plant individually.

2.4. Statistical analysis

The experimental design was a randomized complete block. Statistical analysis was performed by the SPSS 11.5 software. The data were analysed using ANOVA and subsequent comparison of means was performed using the Fisher's LSD test at $p < 0.05$.

3. Results and discussion

3.1. Nodulation test

3.1.1. Number and nodule biomass

A large variability in nodule number and dry weight was detected among the fifty four rhizobia strains (Table 2). Results showed that inoculation with Ar02 strain revealed a high nodulation (134 nodules $plant^{-1}$) compared with other rhizobia. These results are in agreement with those reported by Abdi et al. (2014). Authors mentioned that Ar02 strain induced the formation of the most nodular number with Coco Blanc variety.

Table 2. Nodulation (number and biomass) inoculated with different rhizobia strains.

rhizobia strains	Nodule number	Nodule dry weight ($g.Pl^{-1}$)	rhizobia strains	Nodule Number	Nodule dry weight ($g.Pl^{-1}$)
Control	0^{j}±0	0^{i}±0	**KHT1.96**	38^{efghi}±8.225	0.01^{fgh}±0
CIAT899	54.75^{cde}±20.726	0.01^{fgh} ±0	**KHT3.96**	0^{j}±0	0^{i}±0
Alia1	0^{j}±	0^{i}±0	**Ar3**	0^{j}±0	0^{i}±0
Alia2.96	89^{b}±15,383	0.02^{cde}±0	**Ar1**	0^{j}±0	0^{i}±0
Tinja	50.75^{cdef} ±15.370	0.017^{def}±0.005	**Ar6**	0^{j}±0	0^{i}±0
Ar02	134^{a}±11.176	0.0375^{a}±0	**Ar4**	0^{j}±0	0^{i}±0
Ar05	50^{cdef}±10.708	0.017^{def}±0.005	**Ar2**	0^{j}±0	0^{i}±0
P.Ar.09	66^{cd}±5.916	0.025^{bc} ±0.005	**S1**	0^{j}±0	0^{i}±0
P.Bj	51.75^{cdef}±	0.02^{cde}±0	**J1.96**	0^{j}±0	0^{i}±0
P.OM.09	51^{cdef}±9.032	0.02^{cde}±0	**J2.96**	32.5^{fghi} ±13.964	0.012^{efgh}±0.005
P.Ps	62^{cd}±16.822	0.03^{ab}±0	**J3.96**	0^{j}±0	0^{i}±0
CB	0^{j}±0	0^{i}±0	**J1.92**	37^{hgfe}±4.690	0.012^{efgh}±0.005
P.Tb	15^{ij}±21.213	0.005^{hi} ±0.005	**J3.92**	66.75^{c}±32.836	0.022^{cd}±0.005

SOM	1.75^{j} ±3.5	0^{i}±0	**J4.92**	0^{j}±0	0^{i}±0
D4.007	0^{j}±0	0^{i}±0	**S3**	0^{j}±0	0^{i}±0
D4.002	0^{j}±0	0^{i}±0	**S7**	3^{j}±5.5	0.002^{cde}±0.005
KHS1	28.25^{ghi}±17.173	0.012^{efgh} ±0.005	**S9**	0^{j}±0	0^{i}±0
KHS2	5.25^{j} ±5.560	0.007^{ghi} ±0.005	**S11**	1^{j}±2	0.001^{i}±0.002
GB.92	47.75^{cdefg} ±6.184	0.02^{cde} ±0	**Raf. Raf**	0^{j}±0	0^{i}±0
GB.258	43.75^{defg} ±7.182	0.017^{def}±0.005	**Ras. JB**	44.75^{defg}±4.27	0.015^{defg}±0.005
KH28	4.5±5.259	0^{i}±0	**Soudan1.2**	2^{j}±2.309	0^{i}±0
Fr1.97	0^{j}±0	0^{i}±0	**Soudan2.2**	0^{j}±0	0^{i}±0
OM	17^{hij}±12.675	0.01^{fgh}±0	**D2.2**	0^{j}±0	0^{i}±0
Mat.94	0^{j}±0	0^{i}±0	**D3.2**	0^{j}±0	0^{i}±0
Zaar	2.75^{j} ±5.5	0.002^{i}±0.005	**Artn1**	0^{j}±0	0^{i}±0
ZG.96	0.25^{j} ±0.5	0^{i}±0	**Ic.208**	0^{j}±0	0^{i}±0
B155	0^{j}±0	0^{i}±0	**12a3**	0^{j}±0	0^{i}±0
S10	0^{j}±0	0^{i}±0	**YH15**	0^{j}±0	0^{i}±0

Data are the means ± SD of four replicates harvested at flowering stage $p < 0.05$

3.1.2. Biomass production

The results of biomass production assays are shown in Table 3.

Table 3. Shoot and root dry weight of common bean genotype inoculated with different rhizobia strains

rhizobia strains	Shoot dry weight ($g.Pl^{-1}$)	Root dry Weight ($g.Pl^{-1}$)	rhizobia strains	Shoot dry weight ($g.Pl^{-1}$)	Root dry weight($g.Pl^{-1}$)
Control	0.277^{d}±0.086	0.1^{cd}±0.045	**KHT1.96**	0.357^{abcd}±0.052	0.142^{abcd}±0.022
CIAT899	0.405^{abcd}±0.045	0.112^{bcd}±0.047	**KHT3.96**	0.312^{abcd}±0.063	0.12^{bcd}±0.014
Alia1	0.385^{d}±0.041	0.17^{c}±0.037	**Ar3**	0.355^{abcd}±0.064	0.162^{cd}±0.022
Alia2.96	0.465^{ab}±0.106	0.13^{bcd}±0.05	**Ar1**	0.305^{abcd}±0.023	0.145^{cd}±0.033
Tinja	0.42^{abcd}±0.129	0.18^{abcd}±0.018	**Ar6**	0.352^{abcd}±0.037	0.117^{cd}±0.015
Ar02	0.445^{abc}±0.093	0.097^{cd}±0.012	**Ar4**	0.351^{abcd}±0.02	0.099^{cd}±0.01
Ar05	0.437^{abc}±0.047	0.0197^{abc}±0.017	**Ar2**	0.35^{abcd}±0.095	0.095^{cd}±0.035
P.Ar.09	0.4^{abcd}±0.115	0.13^{bcd}±0.024	**S1**	0.366^{abcd}±0.056	0.12^{bcd}±0.03
P.Bj	0.407^{abcd}±0.056	0.115^{bcd}±0.03	**J1.96**	0.277^{d}±0.034	0.107^{cd}±0.027
P.OM.09	0.342^{bcd}±0.076	0.14^{bcd}±0.031	**J2.96**	0.345^{bcd}±0.058	0.107^{cd}±0.04
P.Ps	0.505^{a}±0.093	0.145^{abcd}±0.033	**J3.96**	0.346^{d}±0.023	0.115^{bcd}±0.046
CB	0.267^{d}±0.067	0.112^{cd}±0.02	**J1.92**	0.395^{d}±0.054	0.117^{bcd}±0.027
P.Tb	0.39^{abcd}±0.078	0.155^{abcd}±0.036	**J3.92**	0.382^{abcd}±0.033	0.112^{bcd}±0.02
SOM	0.362^{abcd}±0.122	0.14^{bcd}±0.029	**J4.92**	0.387^{d}±0.082	0.102^{cd}±0.017
D4.007	0.307^{bcd}±0.045	0.145^{abcd}±0.038	**S3**	0.365^{abcd}±0.160	0.267^{cd}±0.12
D4.002	0.355^{d}±0.,083	0.16^{cd}±0.03	**S7**	0.357^{abcd}±0.072	0.137^{bcd}±0.04
KHS1	0.272^{d}±0.074	0.152^{abcd}±0.034	**S9**	0.297^{d}±0.089	0.177^{cd}±0.038
KHS2	0.32^{bcd}±0.094	0.137^{bcd}±0.06	**S11**	0.362^{abcd}±0.203	0.227^{bcd}±0.19
GB.92	0.425^{abcd}±0.103	0.12^{cd}±0.053	**Raf .Raf**	0.37^{d}±0.073	0.122^{d}±0.023
GB.258	0.362^{abcd}±0.080	0.07^{d}±0.04	**Ras.JB**	0.352^{abcd}±0.068	0.14^{bcd}±0.024
KH28	0.375^{abcd}±0.081	0.102^{cd}±0.03	**Soudan1.2**	0.31^{bcd}±0.041	0.105^{cd}±0.02
Fr1.97	0.297^{d}±0.061	0.095^{cd}±0.017	**Soudan2.2**	0.357^{abcd}±0.123	0.2^{cd}±0.020
OM	0.345^{bcd}±0.075	0.0137^{bcd}±0.033	**D2.2**	0.312^{abcd}±0.038	0.185^{abcd}±0.03
Mat.94	0.22^{d}±0.046	0.117^{cd}±0.027	**D3.2**	0.307^{bcd}±0.076	0.157^{abcd}±0.09
Zaar	0.287^{cd}±0.056	0.09^{cd}±0.021	**Artn1**	0.352^{abcd}±0.092	0.13^{abcd}±0.049
ZG.96	0.295^{cd}±0.071	0.122^{bcd}±0.026	**Ic.208**	0.350^{abcd}±0.012	0.17^{abcd}±0.032
B155	0.365^{d}±0.028	0.19^{cd}±0.049	**12a3**	0.372^{bcd}±0.068	0.125^{abcd}±0.034
S10	0.275^{d}±0.019	0.13^{cd}±0.038	**YH15**	0.392^{bcd}±0.116	0.14^{cd}±0.014

Data are the means ± SD of four replicates harvested at flowering stage $p < 0.05$

Shoot dry weight was improved by rhizobia strain inoculation. Statistical analysis of the results showed that Ar05, Ar02, Alia2.96 and P.Ps are the efficient rhizobia strain and biomass production varied between 0.27 $g.Pl^{-1}$ to 0.50 $g.Pl^{-1}$ (Table 3). Root dry weight production is influenced by rhizobia inoculation S3, S11, Ar05 and Tinja strains improved root growth with production of biomass which is 0.27g.Pl^{-1} (Table 3). Accordingly, Zaman- Allah et al. (2007) and Abdi et al. (2012) have demonstrated that vegetative growth response depends on the rhizobial inoculation and common bean variety.

3.1.3. Nitrogen content

Variation of the nitrogen content in shoot is shown in table 4. The nitrogen content isin order to 2.85% in plant inoculated with J3.92. Control plants have low nitrogen content about 1.85%. Inoculation with rhizobia strain (Ar02) increased the nitrogen content to 1.91% and the increase was in order to 45% compared to control plants (Table 4). It has been reported that nitrogen fixation increased significantly with rhizobia strain (Khan et al. 1997)

Table 4. Nitrogen content of common bean plants inoculated with different rhizobia strains.

Rhizobia strains	Nitrogen Content%	Rhizobia strains	Nitrogen content %
Control	1.85^{d}±0.221	**KHT1.96**	2.83^{a}±1.148
CIAT899	1.94^{cd}±0.138	**KHT3.96**	1.6^{e}±0.272
Alia1	1.19^{g}±0.241	**Ar3**	1.55^{ef}±0.132
Alia2.96	2.08^{c}±0.028	**Ar1**	1.89^{cd}±0.017
Tinja	2.44^{b}±0.830	**Ar6**	1.94^{cd}±0.269
Ar02	1.91^{cd}±0.247	**Ar4**	1.92^{cd}±0.03
Ar05	2.33^{bc}±0.606	**Ar2**	1.53^{ef}±0.080
P.Ar.09	1.66^{de}±0.186	**S1**	2.01^{c}±0.05
P.Bj	1.96^{cd}±0	**J1.96**	1.66^{e}±0.258
P.OM.09	1.56^{de}±0.378	**J2.96**	2.47^{b}±0.456
P.Ps	1.92^{cd}±0.057	**J3.96**	2.85^{a}±0.08
CB	1.63^{de}±0.23	**J1.92**	2.14^{c}±0.045
P.Tb	1.67^{de}±0.243	**J3.92**	2.85^{a}±0.380
SOM	1.85^{d}±0.591	**J4.92**	1.27^{f}±0.023
D4.007	1.49^{de}±0.23	**S3**	1.67^{e}±0.080
D4.002	1.94^{cd}±0.269	**S7**	1.73^{de}±0.028
KHS1	1.80^{d}±0.456	**S9**	1.56^{ef}±0.432
KHS2	2.02^{c}±0.423	**S11**	1.66^{e}±0.235
GB.92	2.01^{c}±0.271	**Raf .Raf**	1.49^{ef}±0.09
GB.258	2.73^{ab}±0.109	**Ras.JB**	2.34^{bc}±0.210
KH28	1.66^{de}±0.186	**Soudan1.2**	1.89^{cd}±0.210
Fr1.97	1.71^{de}±0.236	**Soudan2.2**	1.25^{fg}±0.126
OM	2.09^{c}±0.460	**D2.2**	1.82^{d}±0.120
Mat.94	1.61^{de}±0.028	**D3.2**	1.47^{ef}±0.484
Zaar	1.32^{f}±0.338	**Artn1**	1.63^{e}±0.23
ZG.96	1.32^{f}±0.138	**Ic.208**	1.45^{ef}±0.210
B155	1.46^{ef}±0.235	**12a3**	1.34^{f}±0.213
S10	2.48^{b}±0.263	**YH15**	1.94^{cd}±0.269

Data are the means ± SD of four replicates harvested at flowering stage $p < 0.05$

3.2. Effect of inoculation and P fertilization on the rhizobia-common bean symbiosis under field conditions

3.2.1. Number and nodule biomass

The results showed that inoculation with CIAT899 and Ar02 strains improved nodulation which reached 8 and 5 nodules /Pl. in Oued Beja and 1 nodules /Pl. in Oued Meliz. The contribution of phosphorus in plants inoculated with CIAT899 and Ar02 improved nodulation of common bean plants (Table 5). Phosphorus increased the number of nodules from 8 to 40 nodules / Pl in Oued Beja and from 1 to 10 nodules / Pl in Oued Meliz (Table 5). The same phenomenon has led to the improvement on shoot and root dry weight. These results suggest that the strain CIAT899 is suitable for common bean cultivation in the region of Oued Beja and Oued Meliz. According to (Bargaz et al. 2012; Abdi et al. 2014), phosphorus fertilization increases the nodular number and plant production. Increasing the number and biomass of nodules under phosphorus fertilization has been reported by Abdi et al. (2014). Effect of phosphorus on nodulation remains partly bound to the high demands for the development of

ATP and the nodular operation (Ribet et al. 1995; Hmissi et al. 2015). The variation of nodulation parameters could be due to the efficiency in acquisition of P from the rhizosphere (Bargaz et al. 2012).

Table 5. Effect of P fertilization and rhizobia strain inoculation on nodulation (nodules number and nodules dry weight) of common bean under rainfed conditions in Oued Beja and irrigated conditions in Oued Meliz.

	Oued Béja		**Oued Méliz**	
Treatments	**Nodule number**	**Nodule dry weight ($mg.Pl^{-1}$)**	**Nodule number**	**Nodule dry weight ($mg.Pl^{-1}$)**
Control	11.5^{b}±11.160	30^{ab}±0.016	1.83^{b}±1.376	2.3^{b}±0.002
P	24.123^{ab}±0.125	37.3^{ab}±0.027	0.87^{b}±0.375	3.33^{b}±0
CIAT899	7.66^{b}±3.60	38.3^{ab}±0.006	1.25^{b}±0.25	1^{b}±0
Ar02	4.59^{b}±1.376	18.3^{b}±0.006	0.62^{b}±0.375	3.33^{b}±0
CIAT899 +P	40^{a}±25.5	50^{a}±0.017	10.14^{a}±7.024	7.33^{a}±0
Ar02 +P	16.92^{ab}±11.364	27^{ab}±0.013	2.5^{b}±0.25	2^{b}±0

Data are the means ± SD of four replicates harvested at flowering stage $p < 0.05$

3.2.2. Growth production

The efficiency of the strain in biomass production is attributed to its power of atmospheric nitrogen. The addition of phosphorus did not improve the biomass production of common bean. These results confirm that inoculation with strains CIAT899 and Ar02 and application of phosphorus had no significant effect on shoot dry weight of common beans in the two sites (Tab.6). Phosphorus intake and inoculation with Ar02 strain improved shoot dry weight. It was in order to 3.56 g / Pl in Oued Beja and 8.06 g /Pl in Oued Meliz. While root dry weight was respectively, 0.3 g /Pl and 0.5 g / Pl in Oued Beja and in Oued Meliz. Phosphorus did not increase the root dry biomass production. The effect of inoculation and the phosphorus apply on root production is comparable to those obtained with control plants in both regions. The effect of other treatments of inoculation and application of phosphorus on root dry matter production is comparable to those obtained with the control plants in both regions. This result is the opposite of that found by (Bargaz et al. 2012; Abdi et al. 2014).

Table 6. Effect of nitrogen fertilization and rhizobia strain inoculation with on biomass production of common bean ($g.Pl^{-1}$) under rainfed conditions in Oued Beja and irrigated conditions in Oued Meliz.

	Oued Béja		**Oued Méliz**	
Treatments	**Shoot dry weight ($g.Pl^{-1}$)**	**Root dry weight ($g.Pl^{-1}$)**	**Shoot dry weight ($g.Pl^{-1}$)**	**Root dry weight ($g.Pl^{-1}$)**
Control	3.31±0.526	0.28±0.014	7.84±0.379	0.47±0.075
P	2.44±0.520	0.23±0.052	7.4±0.6	0.42±0.075
CIAT 899	3.88±0.608	0.27±0.066	8.32±1.872	0.49±0.115
Ar02	3.71±0.482	0.29±0.072	8.83±1.945	0.56±0.072
CIAT899 +P	3.85±0.,646	0.32±0.066	9.75±1.803	0.62±0.114
Ar02 +P	3.56±0.625	0.3±0.025	8.06±0.775	0.5±0.05

Data are the means ± SD of four replicates harvested at flowering stage $p < 0.05$

3.2.3. Nitrogen content

Control plants have a low nitrogen content that is in order to 1.71 % in Oued Béja, while the contribution of phosphorus and/or rhizobia inoculation increased the nitrogen content to 2.1% (Table 7). The addition of phosphorus fertilization in plants inoculated by rhizobia strain (Ar02) has no significant difference on the nitrogen content compared to control plants. Under field conditions, the reduced growth and nodulation emphasized with significant variations in N content. Control plants have a low nitrogen content that is in order to 1.71 % in Oued Béja, while the contribution of phosphorus and /or rhizobia inoculation increased the nitrogen content to 2.1 %. The addition of phosphorus fertilization in plants inoculated by rhizobia strain has a minor effect on the nitrogen content compared to control plants. In comparison with other legume, (Vadez et al. 1996; Zaman et al. 2006) confirm the high sensitivity of symbiotic nitrogen fixation to the type of fertilization in common bean.

Table 7. Effect of phosphorus fertilization and rhizobia strains inoculation on nitrogen content in common beans under rainfed conditions in Oued Beja and irrigated conditions in Oued Meliz.

	Oued Béja	Oued Meliz
Treatments	**Nitrogen content (%)**	**Nitrogen content (%)**
Control	1.71±0.072	2.14±0.266
P	2.1±0.076	1.92±0.109
CIAT 899	1.91±0.115	2.47±0.285
Ar02	1.94±0.108	1.92±0.130
CIAT899 +P	2.1±0.061	2.3±0.285
Ar02 +P	1.79±0.051	2.3±0.045

Data are the means ± SD of four replicates harvested at flowering stage $p < 0.05$

3.2.4. Grain Yield and weight of 100 seeds

The seeds were harvested at crop maturity in Oued Beja and Oued Meliz stations from the two central rows of each block in order to assess the grain yield. One hundred seeds from each set were also weighted in order to estimate seed weight. In Oued Meliz, inoculation with Ar02 and additional P fertilizer led to an increase in grain yield and produced high weight seeds but it is lower in Oued Beja (Table 8). In Oued Meliz, inoculation with Ar02 and P fertilization increased yield and weight of seeds respectively from 11.67 to 15.33q/ha and from 20.96g/100 seeds to 23.33g/100 seeds (Table 8). In addition, the yield grain and weight of 100 seeds during season 2012/2013 were improved following inoculation with Ar02 and CIAT899 strains and the contribution of phosphorus fertilizers supply. Several studies have reported a positive effect of inoculation leading to an improvement in seed yield (Abdi et al. 2014). Although, a small contribution to crop production compared to fertilization, the amount of symbiotic nitrogen fixation remains very useful in maintaining and restoring soil fertility.

Table 8. Grain yield and weight of 100 seeds of Coco Blanc variety of Common bean under rainfed conditions in Oued Beja and irrigated Oued Meliz.

	Oued Béja		Oued Méliz	
Treatments	**yield (q/ha)**	**100 seeds(g)**	**Yield (q/ha)**	**100 seeds(g)**
Control	6.06±2.07	23.73±1.62	11.67±1.66	20.96±1.62
P	5.59±0.54	23.30±0.66	11.67±0.86	23.94±0.66
CIAT899	4.82±1.94	23.27±0.81	8.10±0.41	22.4±0.81
Ar02	6.18±1.06	23.87±0.82	10.50±1.26	21.58±0.82
CIAT899+P	6.03±1.03	22.80±3.41	11.00±1.48	23.21±3.41
Ar02+P	6.77±2.77	25.27±2.17	15.33±2.34	23.33±2.18

Data are the means ± SD of four replicates harvested at flowering stage $p < 0.05$

The present work aims to evaluate the importance of rhizobia inoculation and the effect of P fertilization on common bean production. Variability in results was showed in response of common bean crop to inoculation and mineral fertilizers. Combination of rhizobial inoculants and P fertilizer revealed an effect on nodulation, growth biomass production, nitrogen content and grain yield. Assessment of strain's infectivity potential showed large variability between the tested rhizobia strain and Ar02 showing a high nodulation number compared with other rhizobia strain, these results are in agreement with those reported by the results of (Abdi et al. 2014).

4. Conclusion

Phaseolus vulgaris-rhizobia symbiosis exhibited different levels of adaptability under soil conditions and site. Combination of rhizobial inoculants and P fertilizer revealed a strong effect on nodulation, plant biomass, nitrogen content and grain yield. This study provides additional evidence that yields could be improved in common bean by inoculation with appropriate rhizobia and adequate phosphorus applications, although significant variation within and among sites and rhizobia were observed. The observation of an increase in nodule number and in N accumulation for each inoculation by Ar02 strain is to our knowledge the first description of a correlation between nodulation, N_2 fixation and rhizobia inoculation.

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