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Study of culture of six symbiotic cultivars bean and survey of their nodulation under field natural conditions in West Algeria

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ABSTRACT: Leguminous host-plants which develop symbiotic relationships with nitrogen-fixing bacteria are essential for reducing chemical fertilizers use. Thus, the ability of legumes including bean to fix nitrogen from the bacteria such as rhizobia, housing within root nodules could be enhanced because of their particular agricultural and ecological importance. Consequently, we tried in the present work to improve the growth conditions of leguminous plants in particular, common bean in Algeria by increasing and developing the symbiotic interaction relating to bean –rhizobia symbiosis. In this paper results obtained from field experiments conditions have shown difference in plants growth and nodulation.The physical and chemical analysis showed that the loam soil of Oulhaça, with an important rate of organic matter, is the best for bean culture. pH, salt, $CaCO_3$ and phosphorus value were noted. Morphological and symbiotic properties, tested in three reference areas, showed diversity in plants growth and colors in field trials conducted on six genotypes (115, 147, 104, 83, 34 and 29) of common bean (*Phaseolus vulgaris* L.) contrasting in efficacy of using phosphorus in order to estimate their nodulation and their behavior under climatic and pedologic conditions. The variety 104 presented the best rate of nodulation and is more adapted on Oulhaça soil when in the same conditions; the variety 83 presented the lower number of nodules.The presence of indigenous population in soil sample and nodules in roots of *Phaseolus vulgaris* indicate the possibility of getting potentially effective adaptable rhizobial strains that enhance bean productivity. Weak symbiotic properties observed during nodulation status survey might partly be responsible for growth variation and reduction in nodules.
Key words: Bean varieties – root nodules – nitrogen fixation –- *Phaseolus vulgaris-Rhizobium* symbiosis – climatic and soil conditions- .

INTRODUCTION

Symbiotic nitrogen fixation feature is an essential component in cultural systems however, the associated leguminous plants are characterized by low yield and this is explained by sensitivity to biotic and abiotic factors like temperature, drought and soil paucity (Baudoin, 2001) as constraints limiting production. Soil nitrogen is one of the most important regulatory players in the symbiotic interaction between leguminous host-plants and Bacteria Nodulating Legume (BNL) (Griffon, 2006). A better understanding and developing new technologies of this symbiosis would contribute to obtain a healthy environment (Bohlool *et al*., 1992).

Legume plants, such as *Phaseolus vulgaris* L. known as common bean are well characterized for its symbiotic association with BNL, it fixes considerable amounts of atmospheric nitrogen which is incorporated to soil (Tamimi, 2002). Bean is the one of the major leguminous crops grown in the world and its seeds are widely consumed. It is an important legume for human nutrition and a major protein (25%) and calorie source (Anderson, 2003), but its yield remain low to moderate due to its scarce nodulation, chemical fertilizers high inputs (Garcia *et al*., 2004). Yields often too low can be improved by a higher N supply through symbiosis with efficient rhizobial strains (Kaschuk *et al*., 2005; Hungria *et al*., 2000).

Algerian agronomists think that production may increase because climatic and pedologic conditions are highly suitable in littoral areas (Abdelguerfi, 2003); improvement of culture conditions and introduction of new productive varieties may contribute to increase yields. Bean variety diversity was not too important in Algeria, but during the last years, its number is increasing by introduction of new cultivars such as Michelet and

Contender, their adaptability depends on the climatic conditions predominating in cultivated area. Biogeographical position of Algeria and its bioclimatic stage structures including moisture, arid and semi-arid areas, make it very interesting for biological resources (Abdelgherfi, 2003). These areas are characterized by fluctuation in precipitations (Boulbaba *et al*., 2009). For leguminous plants, climatic changes and drought affect not only host plants but also growth and presence of rhizobial population in soil, development and nodules functions and so nitrogen fixation capacity.

Most published works on the ecology of BNL show that microorganisms are affected by changes in soil factors such as moisture, temperature, pH, soil toxicity, nutrient deficiencies and organic matter. The variation in nodule number and color induce variation in growth and yield of the host due to variation in fixed nitrogen (Lupwayi and Mkandawire, 1996; Amijee and Giller, 1998).

In Mediterranean regions, bean production is limited by soil phosphorus deficiency (Shen *et al*., 2001). The total percentage of phosphorus (P) in soil is approximately around 0.04 to 0.10%, but only 1.00 to 2.50% can be absorbed by plants. The low availability of phosphorus nutrition in soils has became the ‘‘limiting factor’’ for root growth (Kanako *et al*., 2004).

In this context, we aimed in this work to compare between three reference areas showing differences in bean plants development and nodulation. It seems that nitrogen fixation is the major factor limiting vegetal growth with possible implication of phosphorus biodisponibility. Furthermore, we tried in the present study to evaluate the ability of this legume crop to induce the endosymbiotic association with root nodulating bacteria (*Rhizobia*) group and to estimate the symbiotic effectiveness of native *Rhizobium* strains on number of nodule and morphological properties of dry bean under field conditions. We compare between six varieties of common bean, tolerant and sensitive to phosphorus deficiency (variety 115, 147, 104, 83, 34 and 29), observe and evaluate their nodulation potential.

MATERIALS AND METHODS

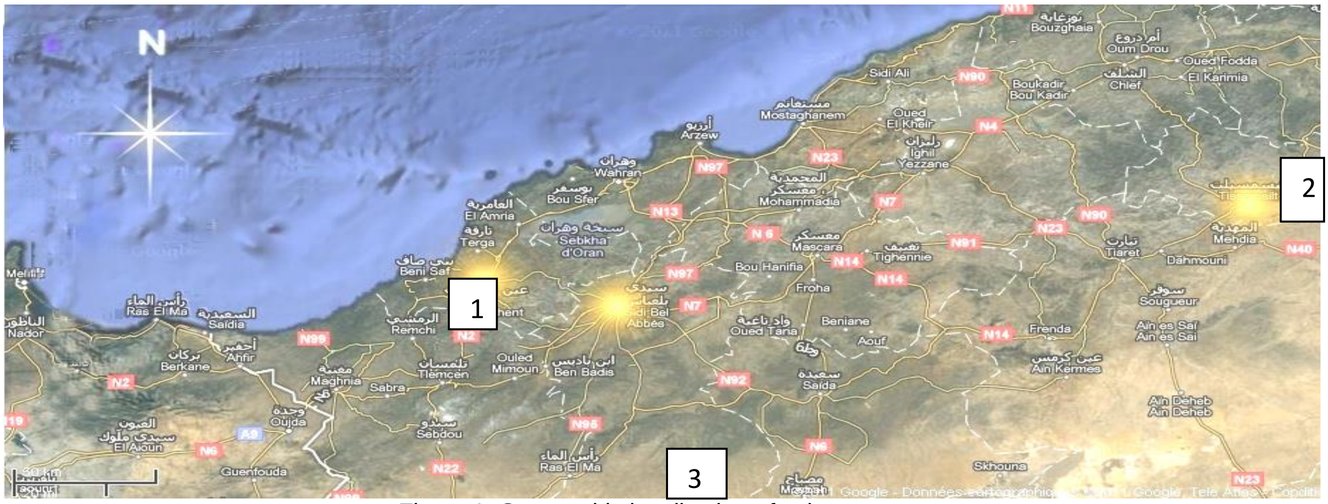
Description of experimental zones

The experimental sites location are indicated in table1.Oulahaça (Beni-Saf in Ain Temouchent), is an area of 10 Hektars and belong to a private farmer. The soil is dark and the ancient culture was carotts. The climate is semiarid with cold moist winters and hot dry summers.

The second zone is situated in Tissemsilt. The soil is light brown rich in fine elements and covered with grass. The third one is situated in Sidi Bel Abbes in the experimental station INRAA (Institut National de Recherche Agronomique). The soil is use for legumes culture; it is light brown and left fallow.

Table 1. GPS data experimental sites

Oulhaça (Beni-Saf Ain Témouchent	Latitude	35° 14’ 35’’ N
	Longitude	01° 26’ 83’’ E
	Altitude	150 m
Tissemsilt	Latitude	50°35’48’’ N
	Longitude	01°49’28’’ E
	Altitude	840 m
Sidi Bel Abbes	Latitude	35° 10’ 35’’ N
	Longitude	00° 38’ 52’’ W
	Altitude	490 m



Figyre 1. Geographic localization of culture zones
Oulhaça (Beni-Saf Ain Témouchent) 1 ; Tissemsilt 2 ; Sidi Bel Abbes 3.

Soil sampling
The soil samples were collected from the field at 20 cm of depth.

Physicochemical analyses

Soil particle size analysis was obtained using Robinson pipette.The pH and electrical conductivity (EC) were estimated in soil water extract using a glass electrode pH meter and an EC meter in 1:5 soil water suspension. The available P was measured using acid extraction method (Duchaufour, 1959). Thus the organic matter of the soil, carbon and $CaCO_3$ were also determined.

Quantification of the number of total indigenous microbial flora

The number of total indigenous microbial flora in the experimental soil samples was determined by serial dilution. 0,1 ml aliquot of each dilution was spreading on solid nutritive medium then incubated at 28°C during 24 h. The results were expressed as CFU number after counting the colonies number (30 and 300).

Climate data

The climate data could give lot of information about relation between climatic conditions and cultures development. Oulhaça area is situated in moist bioclimatic stage referring to Emberger climagram based on the five last years. This region show a dry period from April to October with annual minimal precipitation of 1,78mm, annual maximal temperature of 26,93°C, a moist period from October to April with maximal annual precipitation 55,50mm and minimal annual temperature 13,03°C.

Tissemsilt area show a moist period from October to May with annuel maximal average precipitation of 47 mm and annual minimal temperature of 5.92 C°. The dry period is from May to October with annual minimal precipitation of 4.8 mm and annual maximal temperature of 26.35 C°.

The dry period in Sidi Bel Abbes is from March to half November and the moist period is from half November to the end of February. This area is characterized by maximal precipitation of 58.5 mm in November and minimal of 2.8 mm in July. The dry period seems took 8 months. The annual average temperature is 26.72 C° in July and 9.21C° in January.

Field experimentation

Tested cultivars

Comparatives test were made on six varieties of bean *Phaseolus vulgaris* contrasting in efficacity of the use of phosphorus (EUP) for their nitrogen symbiotic fixation (FSN): 115, 147, 104, 83, 34 and 29, these cultivars were identified from a hybrid of BAT 477 and DOR 304 cultivars. The molecular and physiological characterization of these genotypes and their ecological interest are studied by the CIAT program (International Center of Tropical Agricultures, Colombia). The varieties are black, tolerant 115; beige, sensitive 147; brown, tolerant 104; beige, sensitive 83; black, tolerant 34; beige, sensitive 29. All seeds were gracefully furnished by the INRA laboratory SupAgro UMR 1222 Eco and Soils.

Test Protocol

As defined by the Groupe Coopératif de Recherche sur les Légumineuses dans le Bassin Méditerranéen FABAMED, seedling was done by putting 12 seeds per line. The distance between two seeds was about 10 cm when the depth seedling was 3 cm. Plants were usually irrigated and trials were conducted in natural conditions. The plot was prepared for seedling and weeding was hand operated.

Observation and notation

During the vegetative plant cycle, main stages developments were noted and agrochemical state was periodically following. All plants were carefully unearthed at flowering stage to look for nodules on roots. Shoots height and leaf colors were noted. The number and size of nodules were determined after a gently wash of roots with water.

RESULTS AND DISCUSSION

Soil physical and chemical analyses

The physical analyses of the experimental soils showed differences in texture (table 2). The soil 1 is moderately alkali and non saline. This soil showed an important quantity of organic matter (Org. Mat) and 5,23% of organic carbon content. Total $CaCO_3$ was 0,8 % and phosphorus was 0,100 mg/l (counted in P_2O_5).

Table 2. Physical and chemical soils result analyses

Soils	pH	CE m/S	total $CaCO_3$	actif $CaCO_3$	Carbon	Org. Mat	Texture
Soil 01	8.22	0.05	0.8 %	-	5.23%	10 %	Sand 40 % Loam 40 % Clay 20 %
Soil 02	7.31	0.15	21%	3.37%	1.23%	2.46 %	Sand 50 % Loam 38% Clay 12 %
Soil 03	8.85	0.2	13.2 %	6 %	1.05 %	2.1 %	Sand 28 % Loam 45 % Clay 27 %

Soil 01: Oulhaça, Soil 02: Tissemsilt , Soil 03: Sidi Bel Abbes

The physicochemical parameters allow appreciation of soil natural fertility, to explain the yield deficiency and to orient choice of cultures (Soltner, 2005). The results show that the soil 1 is loam in texture and in general, the root system of leguminous like common bean is weak and situated not very deep in soil.

Roots development depends also of some conditions like pH (Baize, 1988; Soltner, 1992). Common bean growth optimally until pH 8 (Hansen, 1994). Salt stress is one of the most important factors limiting production of leguminous particularly when nitrogen nutrition depends on symbiotic fixation. Based on the physical results, the Oulhaça study area seems to be a good site for leguminous culture.

Soil 2 is sandy-loam and soil 3 is silty clay. Both soils are poor in carbon content and organic matter which indicate low microbial activities (Robert, 1996) in comparison with Oulhaça soil. Organic matter recorded there, showed that the soil is of good quality. Structurally, when carbon is present, soil is more stable (Robert, 1996) for agricultural and environmental functions.

According to Baize standards (1988), the presence of $CaCO_3$ limited the number of nodules. In the temperate zones of West Algeria, cations are not uptake under hard evaporation compound to less precipitation.

Phosphorus is very important to plants at young stages and it resulted in significant difference for all morphological parameters. Its quantity may be not sufficient because Oulhaça soil is naturally devoid, or its reserves were gradually exhausted by successive cultures. Phosphorus deficiency affect the growth of bean plants, it reduces shoot growth to the detriment of root growth giving less and little nodules (Ribet and Drevon 1996). Limiting nodulation recorded in this study can be attributed to phosphorus deficiency wich increase nitrogen content in soil as reported by Jebara *et al*. (2005). This result underlines the essential role of phosphorus to *Phaseolus vulgaris –Rhizobium* symbiosis, an interesting way to produce common bean in poor lands (Zaman *et al*., 2004).

Olivera *et al*. (2004) showed the impact of phosphorus nutrition on plant growth, symbiotic nitrogen fixation, ammonium assimilation carbohydrate and amino-acid accumulation as well as nitrogen, phosphorus and ATP content in tissues in common bean (*Phaseolus vulgaris*) plants. Braschi *et al*. (2003) reported that different rates of organic matter addition increased extractable phosphorus at different soil-moisture regimes by inhibiting phosphorus insolubilization,

Total bacterial flora

The count of total flora in the experimental soils indicated that the population of native microorganisms is more important in Oulhaça (162x 10^3 CFU/g) than Tissemsilt (116x 10^3 CFU/g) and Sidi Bel Abbes (50x 10^3 CFU/g). Soil fertility favors all microbial activities where BNL strains must be able to compete efficiently with the other microorganisms and resist to antagonists (Hoang and Diem, 1993). The abundance of microorganisms can be in relation with organic matter and in accordance to Thomas and Mbina (2007), principal conditional factors of microbial number and diversity include soil composition, pH, moist and depth which allow also finding correlation between the presences or not of BNL. In another way, this is due largely to the non-culturability of most microbial cells and also to problems of extracting microorganisms from soil. The effects of climate on soil development are largely due to temperature and precipitation which vary considerably across climatic zones. The climate changes in Oulhaça, both temperature and precipitation, may influence the abundance of some microbial groups and potential increase in microbial biomass resulting on change of vegetation and consequently on soil biological properties.

Field observation

The vegetative cycle showed sprouting after more than a week. Flowering was recorded one month later. Our field observation showed that the varieties grow very well in Oulhaça soil after 7 to 10 days of germination of seeds. Measured parameters are given in table. 3.

Table 3. Plant growth characteristics of varieties in field (Oulhaça)

Bean variety	Num. of germinated seeds	Emergence (days)	Shoot length (cm)	Leaves lengh (cm)	Leaves width (cm)	Leaves color	Nodules number	Nodules size (mm)
147	7	10	14.5	6.8	5.6	Green	11	1 - 4
115	8	9	16.2	8.7	6.2	Green	11	2 – 3
104	8	9	14.5	8.8	6.8	Green	35	1- 2
83	8	11	14.6	8.5	6.6	Green	2	1
34	10	9	18	8.9	6.2	Green	8	1 – 3
29	8	10	17	8.2	6.3	Green	9	1 – 3

Table 3 . Plant growth characteristics of varieties in field (Tissemsilt)

Bean variety	Num. of germinated seeds	Emergence (days)	Shoot length (cm)	Leaves lengh (cm)	Leaves width (cm)	Leaves color	Nodules number	Nodules size (mm)
147	9	10	15.5	7.7	6.3	Light green	21	1
115	10	9	14.2	8.2	7	Light green	14	1 – 1.5
104	7	109	16.2	9	7.2	Light green	5	1
83	11	11	16.7	8.5	7.6	Light green	2	1.5
34	8	11	15	9.8	6	Light green	31	1
29	11	10	16.3	7.3	6.2	Light green	3	1

Table 4. Plant growth characteristics of varieties in field (Sidi Bel Abbes)

Bean variety	Num. of germinated seeds	Emergence (days)	Shoot length (cm)	Leaves lengh (cm)	Leaves width (cm)	Leaves color	Nodules number	Nodules size (mm)
147	12	9	13.4	7.5	6.5	Green	00	-
115	8	9	20.2	8.3	6.3	Green	00	-
104	8	9	26	7.6	4.7	Green	00	-
83	12	10	17.4	8.5	6.6	Light green	00	-
34	10	9	14.5	7.5	5	Green	00	-
29	11	10	19	9.2	6.5	Light green	00	-

Because of climatic changes these last years, the temperature influence highly the vegetative plant stages and as cited by Kolef (1974), common bean seeds do not germinate under to +10°C and the best germination is observed between 31,8° C and 33°C ; the limited temperature of germination is 48°C witch situate Oulhaça in a good position for culture of bean. Bean flowering begins 30 to 60 days after seedling according to varieties, soil and climatic conditions (Kolef, 1974), the variety 104 is most adapted on this type of soil under dry period and little water. Similar result was given by Boulbaba *et al*. (2009) and by Purcell *et al*. (1997) on two cultivars of soy bean.

Nodulation survey

In the three experimental stations, nodules survey shows significant variations at each site and for each variety. Nodules showed significant difference between bean varieties in Ohlhaça. The highest average nodule number was recorded by the variety 104 of common bean plants (fig.2) which developed 35 nodules following by variety 115 and 147 respectively, while in the same soil and under the same conditions, variety 83 recorded the lower number of nodules with 02 nodules.

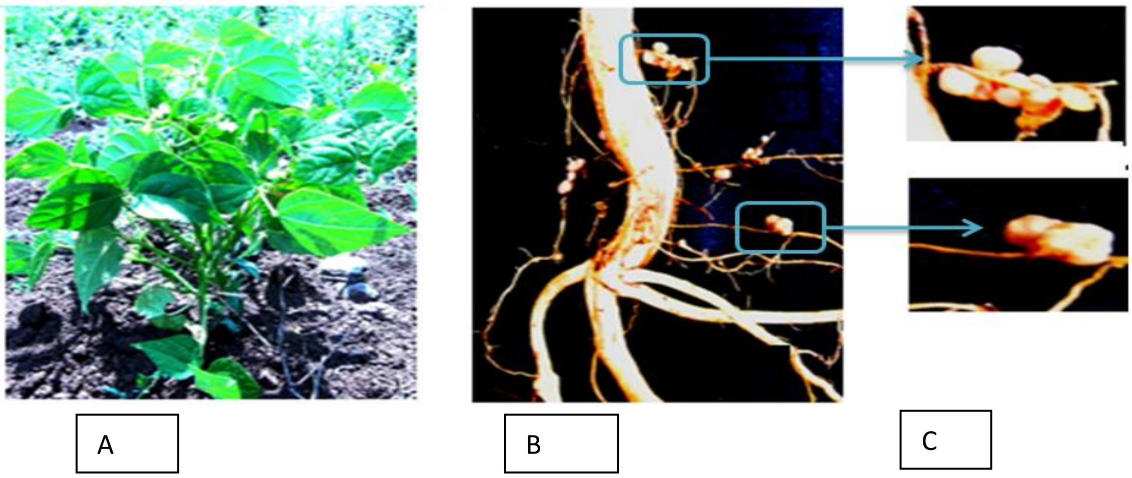


Figure 2 .Nodule aspect of bean variety 104 cultivated in Oulhaça soil
A: Growth of plant B: Root system C: Nodules

Our results suggest the presence of an indigenous rhizobial population because of their capacity to infect and nodulate roots bean of all colored varieties cultivated in this soil which seems very interesting. The difference in nodules number can be explained by different environmental factors (Boulbaba *et al*., 2009) or by difference of potential in nitrogen symbiotic fixation of native partners BNL.

Poor nodulation in our study might indicate the poor plant growth in field. Generally, less nodules produced low grain yields while good nodulation increases N-fixation, growth and yield (Fomeg-As, 2004). It depends in crop rotation system used by the farmers to enhance soil fertility.

Nodulation is totally absent in varieties grown in the resort of Sidi Bel Abbes, the plants are treated with nitrogen fertilizers, they have resulted in an increase in aboveground biomass and decreased nodulation (Boulbaba et al . 2009), which explains the total lack of symbiosis *Rhizobium-Phaseolus vulgaris*. The type of soil can also cause such results, as has been suggested by Jebara *et al*. (2005) who found that edaphic factors significantly affect the number, nodule biomass and absorption of nitrogen. As cited before, the region of Sidi Bel Abbes receive very little rainfall and the soil is poor in nutrients. These unfavorable conditions prevent the development of microbial flora in the soil essential for growth and plant nutrition (Hatimi, 2001).

Yield reduction can be improved through inoculation of adaptable effective rhizobia which improve nodulation and yield referring to Lupwayi and Mkandawire (1996) and Aynababa *et al*. (2001). Therefore, field inoculation trail, survey on environmental factors responsible for poor nodulation and count on number of indigenous rhizobial populations are recommended for further exploitation of BNF. Yield instability in culture of leguminous in Algeria in general and in Ain Temouchent in particular is due in part to biotic and abiotic constraints such as moisture, variation of temperature and soil deficiency especially in phosphorus. Our experiences showed nodular variability between bean genotypes tested and in order to select *Phaseolus vulgaris-Rhizobium*-soil-climate association, variety 104 present the best growth and nodulation that why tolerance of variety 104 to phosphorus deficiency makes it a tool to detect area for bean culture in reference land like Ain Temouchent.

Future works should focus on native population, effect of climatic changes on symbiosis and nodulation and finally selection of more bean cultivars for the use of available P and N supply to identify rhizobial strains that are capable of establishing symbioses with different grain legumes, examine their contribution to dry matter yield, biological nitrogen fixation and compare the effect of soil type on the grain legumes response to inoculation.

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