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Effects of inoculation by *bradyrhizobium japonicum* strains on nodulation, nitrogen fixation, and yield of *lablab purpureus* in Algeria

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Abstract

With the aim of acquiring a better understanding of ecological growth and biomass of *Lablab purpureus* and to assess the effect of *Bradyrhizobium japonicum* strains on the performance of *Lablab purpureus* and production of Specific biofertilizers in this legume*,* an experiment was conducted during the February 2012 to May 2013 growing season. Inoculation of *Lablab purpureus* with *Bradyrhizobium japonicum* and grew them in a greenhouse for 2 months under varying light (L), phosphorus (P), and nitrogen (N) treatments. I obtained the following results: (1) L, P × N, and L × P × N treatments affected every response variable, but most increases. All the nodulation parameters, namely, nodulation rating, nodule number per plant, nodule volume per plant, and nodule dry weight were significantly influenced by the main effect of *Bradyrhizobium japonicum* strains alone.

The results obtained were analyzed statistically by ANOVA using the software statistica and had shown that the main effect of *Lablab purpureus* was highly significantly. The yield and the yield components such as number of pods per plant, number of seeds per pod, seed yield, thousand seed weight, above-ground dry biomass, and total nitrogen uptake were highly significantly affected by inoculation of *Bradyrhizobium* strains alone. A yield increase of 80% was obtained due to inoculation over the uninoculated control. Effect was also significant on number of pods per plant, seed yield, thousand seed weight, harvest index, and total nitrogen uptake. Variety and *Bradyrhizobium* strain interaction was detected on number of nodules per plant and nodule dry weight.

Keywords: *Lablab purpureus*, inoculated, *Bradyrhizobium japonicum*, analyzed statistically, nodulation.

Introduction

Lablab purpureus (Linn.) Sweet (synonym Dolichos lablab L. ex Sweet), is an herbaceous legume, is a globally important is an herbaceous legume crop and source of high quality protein for human consumption, used as fodder for animal and is also important in improved crop rotation systems (Manyong *et al.,* 1996; Carsky *et al.,* 1997). It is also much cultivated to improve the fertility of the soil (Gao et al., 2001), although very little taxonomic work has been done on the symbiotic bacteria associated with this plant. In China, the genetic diversity of rhizobia associated with *Lablab purpureus* has been revealed in different geographical regions (Gao et al., 2001), Symbiotic bacteria such as *Rhizobium, Bradyrhizobium*, and *Sinorhizobium* belonging to the group rhizobia (from the Latin 'root living') play an important role in the nutrition of leguminous plant by fixing atmospheric nitrogen in root nodules de *Lablab purpureus* (Gao et al., 2001).

When in symbiotic association with *Bradyrhizobium japonicum, Lablab purpureus* plants can fix up to 400 kg N ha −1yr − 1 (Smith & Hume, 1987), reducing the need for expensive and environmentally damaging nitrogen fertilizer. Nodulation of soybean requires specific *Bradyrhizobium* species (Abaidoo et al., 2007). In soils where the *Lablab purpureus* crop has not been grown previously, compatible populations of *bradyrhizobia* are seldom available (Abaidoo *et al.,* 2006). The nitrogen demand of *Bradyrhizobium* can be supplied via biological nitrogen fixation through the inoculation with selected *Bradyrhizobium japonicum*/ *B. elkanii* strains. Biological nitrogen fixation can reduced the need for N fertilizers, resulting in an economy estimated in US$ 3 billion per crop season (Nicolás et al., 2006).

The symbiosis between *Lablab purpureus* and *bradyrhizobia* results from a complex process involving many genes of both partners that leads to the formation of N^2 ⁻fixing nodules in roots (Provorov & Vorob'ev, 2000).

EM (Effective Microorganisms) Technology of nature farming was introduced byJapanese scientists in late 1999 (Higa, 2010). EM culture consists of co-existing beneficial microorganisms, the main being the species of photosynthetic bacteria viz., Rhodopseudomonas plastris and Rhodobacter sphacrodes ; lactobacilli viz., Lactobacillus plantarum, L. casei and Streptococcus lactis ; yeasts (Saccharomyces spp), and actinomycetes (Strptomyces spp.). These microorganisms improve crop growth and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, controlling soil diseases and accelerating decomposition of lignin materials in the soil (Higa, 2000; Hussain et al., 2002). Application of EM is known to enhance crop growth and yield in many crops both the guminous and non-leguminous (Sheng & Lian, 2002; Javaid, 2006 , 2009; Khaliq *et al.,* 2006; Daiss *et al.,*2008). The objective of our study was to study the production of Specific biofertilizers in this legume (*Lablab purpureus*) useful to fertilize the soil and reduce chemical inputs. It may be used in two way or coating before their seeds or seedlings in liquid form during the following planting. This biofertilizer is only non-pathogenic bacteria capable of fertilizing the soil with nitrogen through the ability to reduce atmospheric N2 to NH3 necessary to increase seed production of a plant rich in protein *Lablab purpureus* we want the rehabilitate.

Materials and Methods

2.1. The experımental sıte

The experiment was conducted in three different region in Algeria during the September 2012- December 2013 growing season, The soil from each region has undergone physical-chemical analysis: (granulometry, total carbon, organic matter, pH, and electrical conductivity, determination of available phosphorus and determination of total nitrogen).

2.2. Samplıng

A collection of root nodules from of different plants of Lablab purpureus is carried out at three sampling sites: Mostaganem, Bouira and Bejaia (Figure-1), these areas are located in the bioclimatic floor with semi-arid or rainfall varying between 200-400 mm, the temperature is surrounded of 5°C in Winter and varies from 32°C to 35°C in summer [3].

2.3. Isolatıon and ıdentıfıcatıon of straıns

The nodules are disinfected by immersion in ethanol at 95% for 5 to 10 seconds and then in the sodium hypochlorite at 12° for 3 minutes and rinsed 10 times extensively in sterile distilled water. They are then cut in Petri dishes containing Yeast Extract Agar medium: YEMA. After 4 to 5 days of incubation at 28°C, the colonies obtained are purified by successive sub culturing, examined by photon microscope to check their purity, and then isolates were stored at -80°C with glycerol (1:1 v/v) in eppendorf.

Bradyrhizobium japonicum that were isolated from root nodules of local soybean cultivar JS-335 were grown in the YEM (yeast extract mannitol) broth (HiMedia Lab., Mumbai).

2.4. Symbıotıc characterızatıon

The seeds of *Lablab purpureus* are disinfected with absolute ethanol during 5 to 10 seconds and then in the sodium hypochlorite at 12° for 3 minutes, rinsed thoroughly with sterile distilled water and putting to germinate on Petrie Dishes containing water agar (0.8 %) in the dark at 28°C. After 4 to 5 days, the root lets obtained are transferred into soil of different region.

Three treatments were applicated

Treatment 1: The seeds of *Lablab purpureus* are innoculate with 20 strains isolated from root nodules of *Lablab purpureus* cultivar in different region of Algeria.

Treatment 2: The seeds were inoculated with *Bradyrhizobium japonicum*.

Treatment 3: uninoculated control.

2.5. statıstıcal analyzes

The infectiveness and effectiveness of the different strains were determined after two months of growth. The number and the dry weight of the nodules as well as the dry weight of the aerial parts are analyzed statistically by (ANOVA) with the software Statistica.

2.6. Nıtrogen uptake at mıd flowerıng and maturıty

The plant samples taken at mid flowering from each pot were oven dried at 70°C to a constant weight, ground to pass a 1 mm sieve, and analyzed for their nitrogen concentrations using the modified Kjeldahl method as described by Jackson. Finally, nitrogen uptake was determined as the product of straw yield and the respective nitrogen content in the straw and reported as mg nitrogen/plant.

Representative seed and straw samples were taken separately from each replication and composited treatment wise. Materials were separately air dried and then oven dried at 70°C to a constant weight, ground to pass a 1 mm sieve and were saved for laboratory analysis of seed and straw nitrogen concentrations. The nitrogen in the plant parts was determined by the modified Kjeldahl method as described by Jackson. Seed and straw nitrogen uptakes were determined as the product of seed and straw yield and the respective nitrogen content in the seed and straw, respectively. Total nitrogen uptake was calculated as the summation of seed and straw nitrogen uptake. The inoculated and uninoculated treatments were compared by comparing the nitrogen uptakes by plants in both cases.

Results

3.1. Physıco-chemıcal characterıstıcs of soıls

The results of the soils analyzes of the three studied sites [Table -1] showed that the soils of Mostaganem and Bejaia contain clay elements in a very great quantity which gives them a clay-loam texture. Concerning the soil of Bouira, it contains coarse elements in a very small quantities and an important content of fine elements, which gives it a muddy-sandy texture. These 3 types of soils are classified among the balanced textures (Hung, 1999). The soil samples from the site of Mostaganem and Bouira have a neutral pH and an important content in organic matter. The soil of Bejaia has an acid pH and having a low content in organic matter and a low percentage in available phosphorus. The three samples are rich in calcium carbonate (CaCo3) which shows good calcium content and total assets with the exception of the soil of Bejaia which has a low content in active lime. Depending on the scale of salinity of Hermann (1980) the three samples are considered as non-saline soils.

Nitrogen is the more variable and deficient element and the more in the soil, its content depends on the Percentage of organic matter, of amendments of fertilizer but also bacterial species of nitrogen fixation. In our case, the majority of the soil analyzes showed an average rate in nitrogen N2. The values obtained for phosphors are greater than 0.030 and 0.01 g.kg-1soil respectively (Fago, 2012); this means that the soils are very rich in phosphorus.

3.2. Nodulatıon parameters

The effect of inoculation on the plants heights is highly significant $P<0.005$, which means that the inoculated plants have a better growth than not inoculated plants, the T-test indicates that the ratio of height of the seedlings inoculated / height of seedlings witnesses varies from 2.65 to 5.95 which explains the significant growth of seedlings inoculated regarding to the control with a standard deviation difference of 27.45.

A highly significant effect on the dry weight of the root part to $P<0.005$, meaning that the dry weight of the root part of inoculated plants is more important compared to plants not inoculated. The T-test show wed that the report dry weight of the root part of inoculated plants on the dry weight of the aerial parts of control seedlings varies from 4 to 13, thus, the inoculated plants are significantly more developed than the controls.

No significant difference between the inoculated strains in all growth parameters (height and the dry weight of the aerial parts and orthophosphate). This lack of significant difference in the nodulation is due to the significant variability observed between inoculated plants within the same treatment strain and therefore to the genetic variability of the plant material.

Inoculation of *Lablab purpureus* seeds with *Bradyrhizobium japonicum* strains has resulted in a highly significant ($P\geq 0,05$) increase in nodulation rating as compared to 20 strains isolated from root nodules of *Lablab purpureus* cultivar in different region of Algeria (Figure 1).

3.3. Nıtrogen uptake

The main and interaction effects on nitrogen uptake due to Lablab and rhizobial strains are presented in (Table 2). The nitrogen uptake by Lablab at mid flowering was significantly ($P\leq 0, 01$) influenced by the main effect of strains. The nitrogen uptake at mid flowering stage was also affected highly significantly ($P\leq 0,05$) by the main effect of innoculation by *Bradyrhizobium japonicum* strain and significantly by the effect of innoculation by 20 strains isolated from root nodules of *Lablab purpureus* cultivar in different region of Algeria, inoculated control statically was no significantly (P› 0,01) (Table 3).

The inoculation of *Bradyrhizobium japonicum* strain resulted in the maximum nitrogen uptake (1621.86 mg/plant) at maturity which was significantly higher than 20 strains isolated from root nodules of *Lablab purpureus* cultivar in different region of Algeria and inoculated control (Table 2).

Table 1. Results of physico-chemical analyzes of soil composites of each site

Site	PH	l Limestone Totale	P (Mg\Kg)	Limestone Active (%)	Carbon (%)	N%	P (Mg\Kg)	Limestone Active (%)	Carbon (%)	Regards Organic (%)	Sand (%)	Limon Coarse (%)	Limon End (%)	Clay (%)
Mostaganem	7.7	1,087	0.1257	0.97	25.8	1.3	25.8	1.32	0.64	1.76	12.1	6.76	7	11.6
BOUITRA	7.7	5,091	0.1352	0.01	20.2	0.1	20.2	0.09	1.52	0.76	19	12.54	3.78	4.5
Bejaia	4.6	2,087	4,968	0.58	21.6	0.6	21.6	0.56	0 ,83	1.54	21.5	8.53	6.1	5.5

Table2. Main effects of Lablab purpureus and Bradyrhizobium japonicum strains on dry matter production and nitrogen uptake at midflowering

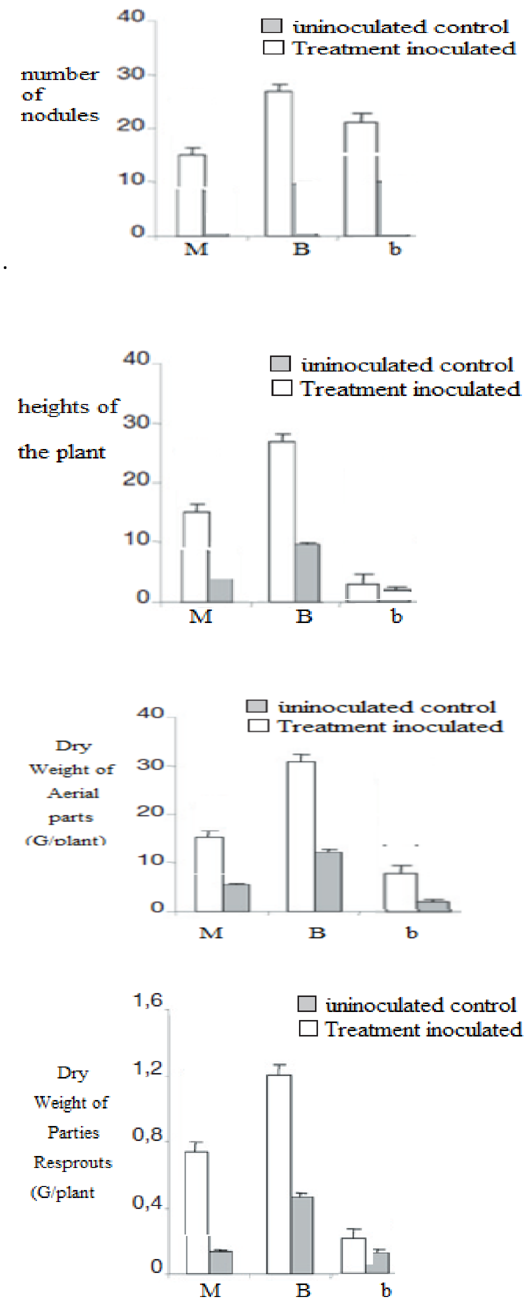
Treatment	Dry matter (g/plant)	Nitrogen uptake (mg/plant)	Significance
DLb1114	18.03b	454.91b	*
DLb 1120	15,55	417.22b	*
Bradyrhizobium	37.12a	1621.86a	**
Tuninoculated control	10.78b	498.12b	NS

NS, **: no significant or significant at P≤0, 01, respectively. Means within a column followed by the same letter(s) are not significantly different.

Table3. Effect of inoculation of *Bradyrhizobium japonicum* on dry weight of shoot and root in *Lablab purpureus*

Treatment	Significance	Shoot dry weight	Rhoot dry weight
20 STRAINS	*	0.71±0.06	0.71±0.06
Bradyrhizobium	**	3.41±2.19	3.89±2.13
uninoculated control	NS	0.05±0.02	0.02±0.009

NS, **: no significant or significant at P≤0, 01, respectively. Means within a column followed by the same letter(s) are not significantly different.



B: The seeds were inoculated with *Bradyrhizobium japonicum*.
b: uninoculated control.

Figure 1. Effect of inoculation on the production strains of the dry matter of the plant Lablab purpureus*M:* The seeds of *Lablab purpureus* are inoculate with 20 strains isolated from root nodules of *Lablab purpureus* cultivar in different region of Algeria.

Discussion

Nitrogen fixation is one of most important biological processes on earth (Graham, 2008) and Rhizobium inoculation of legumes is one of the success stories of world agriculture (Herridge, 2008). The response of inoculum with respect to nodule formation (NOPP)/ root and shoot length and their dry weight was reflected in dry matter production as well.

In this study, increased number of root nodules when compared with control treatments. There is a strong positive relationship between number and dry weight of nodules and yield and per plant in Lablab (Roy and Mishra, 1975; Tiwari and Agrawal, 1977). Tarafdar and Rao (2001), also reported positive interaction between Rhizobium and fungi inoculation on nodulation. In our study, there was a significant difference among isolates over controls was observed. However, the response of inoculation treatment varied with different bacterial strains.

Bradyrhizobium inoculation significantly ($P< 0.05$) increased dry weights, the dry weights of shoots and number of nodule / plant compared to the uninoculated control and 20 strains. These results are in agreement with those reported by Elsheikh, (1998), Okereke and Unaeglu(1992), Salih (2002) , Penfold et al., (1995), Pankhurst et al. (1997) and Schawarz et al., (1999).

Forage legumes have likewise yielded valuable information when treated with rhizobial inoculants: hyacinth bean cultivars were reported to nodulate and were able to fix nitrogen and responded positively when inoculated with competent rhizobial strains, Mahdi and Atabani, 1992,Abril et al., (1997) and Nandasena et al. (2001) also demonstrated that inoculation of soybean with native and well-adapted rhizobia resulted in a better response than inoculation with exotic, non-adapted strains. Rastogi *et al.*, (1981) concluded that there was a significant increase in number of root nodule and dry matter of plants when Rhizobium culture was applied to the seedlings. Similar results were obtained by Abaidoo *et al.*, (2000) with native *Bradyrhizobium* strains where increase in nodulation was observed in host plants. Several workers have demonstrated that inoculation with indigenous *Bradyrhizobium* strains favours the formation of nodules on primary root (Milic *et al.,* 1992; Soliman *et al.,* 1996). Bhat *et al.,* (2011) also reproduce the data on nodulation and revealed that the inoculation with Rhizobium along with fungi significantly increased the nodule dry weight per plant. There was a strong positive correlation between number of root nodules and crop yield which suggest that optimization of root nodulation by inoculating compatible and effective *Bradyrhizobium japonicum* strains significantly increase the soybean crop yield.

Bradyrhizobium japonicum isolates showed significant increase in root length and dry weight. Dhami and Prasad (2009) concluded that inoculation of *Rhizobium* increase the accumulation of N in the root and results in increase in root length and also reported that nitrogen content in soil of inoculated experimental pots increased up to 13-33%.

It is known that indigenous rhizobial populations fix N2 with different efficiency depending of their density and activity and affect the root part and nodulation (Mpepereki et al., 2000; Musiyiwa *et al*., 2005). Therefore, higher availability of nitrogen, significantly increased uptake, dry matter accumulation, translocation of nutrients during reproductive stage which in turn improved yield attributes that affected the grain and straw yield at the end (Patel *et al.,* 1988; Kumar *et al.,* 2002; Sharma *et al.,* 2003).

Conclusion

In the present study, it is clear that inoculation of *Bradyrhizobium japonicum* is showing an increased nitrogen fixation. It is clearly revealed that seedling growth in *Lablab purpureus* can be enhanced by inoculating the seeds with the effective *Bradyrhizobium japonicum* strains.

The results of the present study have indicated that it is important to promote the appropriate use of biofertilizers through national fertilizer programs. efforts should be made, wherever possible, to introduce inoculation technology to the farming community. more research based on standard methods needs to be undertaken to assess the contribution of nitrogen-fixing plants to the overall nitrogen budget. for an alternative use *bradyrhizobium japonicum* strain can be recommended for *lablab purpureus* inoculum production.

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References

Abaidoo,F. 2007. Production of Mungbean (Vigna radiata L.) as affected by nitrogen and phosphorus fertilizer application. In J. Appl. Sci 10:843-847.

Abaidoo, F. 1999. In growth and yield response of mungbean (Vigna radiata L.) to different seed rates and levels of phosphorous. Pak. J. Biol. Sci 2: 879-880.

Abril, S. 1997. Recent developments in Indian agriculture, a state level analysis. In Econom. & Political Weekly 29: 2-18.

Atabani, M. 1992. Effect of Rhizobium inoculation and nitrogen fertilizer on yield and yield components of mungbean. In Int. J. Agric. Biol 2: 238-240.

Abaidoo,F . 2000. In Combined Effects of Rhizobium and Vesicular Arbuscular Fungi on Green Gram (Vigna radiata L. Wilczek) under Temperate Conditions. Research Journal of Agricultural Sciences 2:17-20.

Bhat, T. 2011. Nonspecific staining: its control in immunefluorescence examination of soil. In Science 162: 1012-1014.

Cars, kJ. 1997. Effect of Rhizobium inoculation, phosphorus and molybdenum on yield and its components in mungbean. In Indian Agriculturist 35:67–69.

Dais, S. 2008. Plant growth promoting rhizobacteria: effects on growth and nitrogen fixation of lentil and pea. In Soil Biol 21:511-517.

El sheikh, A. 1998

Fago, Y. 2012. Combined effects of Rhizobium and VA-mycorrhiza inoculation on groundnut. In Journal of Soils and Crops 15:315-318.

Gao, H. 1997. Influence of rhizobacterial inoculation on growth of the sweet potato cultivar. In On Line Journal of Biological Science1 2005:176-179.

Graham, J. 2008. Nodulation competition among Bradyrhizobium japonicum strains as influenced by rhizosphere bacteria and iron availability. In Biology and Fertility of Soils 7: 108–112.

Higa, J . 2010. In A manual of Soil fungi (II eds). Oxford and IBH publishing Co 1956: 220-230.

Hussain, M . 2002. In Statistical procedure for agricultural research. John

Wiley and Sons 17: 368-372.

Hung, R. 1999. Ecological of the root-nodule bacteria of legumes. Nature 9:06-54

Herridge, R. 2008. Hypernodulation of soybean, mung bean, and hyacinth bean is controlled by a common shoot signal. In Crop Science. 37: 1242–1246.

Hayat, S. 2008. Estimation of N2-fixation of mung bean and mash bean through xylem ureide technique under rainfed conditions. In Pak 40:723-734.

Herridge B. 2008. Inoculation technology for legumes. Nature 20: 77–81.

ITO, T. 2008. The autoregulation of nodulation mechanism is related to leaf development. In Plant Cell 49:121–125.

LI, A. 1988. Co-inoculation with antibiotic-producing bacteria to increase colonization and nodulation by rhizobia. In Plant and Soil 108: 211-219.

Millic, S. 1992. Braydrhizobium japonicum capacity to synthesize growth regulators affecting nodulation and nitrogen uptake by soybean. In Mikrobiologija Beograd 8:145-152.