GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

Check for updates

Mycorrhizal fungi inoculation effect on plant growth and phosphorus metabolism of snap bean variety "Contender"

Hayet BELTAYEF [1, 2, *], Wafa SAIDI [1], Rim HAJRI [3], Mouna MECHRI [4] and Mongi MELKI [1]

[1] *Higher School of Agriculture of Kef, Tunisia.*
[2] *Faculty of Sciences of Bizerte, University of Carthage, Tunisia.*
[3] *Regional Research Development Office of Agriculture in Semi-arid North-west of Kef, Tunisia.*
[4] *National Institute of Field Crops, Tunisia.*

GSC Advanced Research and Reviews, 2023, 15(03), 201–206

Publication history: Received on 15 April 2023; revised on 11 June 2023; accepted on 21 June 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.15.3.0156

Abstract

Biofertilizers management for sustainable agriculture is a challenge to improve farmers 'food security today and correct nutrient deficiencies (potassium, phosphorus and nitrogen) in plant.

As biofertilizers, mycorrhizal symbiosis plays an important role in nutrient uptake and the productivity of plants. The aim of this study was the *Rhizobium* and mycorrhizal inoculations effect on plant growth and mineral nutrition of snap bean variety "Contender" in greenhouse condition.

The experiment was a randomized block design with four treatments: a control non-inoculated, dual inoculation with *Serendipita indica* (*S.indica*) and *Rhizobium tropici* Ciat 899 strain (Ciat 899), dual inoculation with *Rhizophagus irregularis* (*R. irregularis*) and Ciat 899, and combined inoculation with *R. irregularis*, *S.indica* and Ciat 899 ; using eight replicates per treatment.

Ciat 899 associated with *S.indica* or *R. irregularis* significantly increased plant growth parameters, and shoots and root phosphorus concentrations. It shows higher biomass production, pod number, nodule number and phosphorus content than plants inoculated with triple strains and non-mycorrhizal plants. Thus, the colonization rate exceeds 80%. The results of this study show that dual inoculation with *Rhizobium* and mycorrhizal fungi enhance significantly productivity in common bean.

Keyword: *Serendipita indica; Rhizophagus irregulari*; *Rhizobium* tropici Ciat 899; Contender

1. Introduction

In Tunisia, some human activities threat the biodiversity. Soils are deteriorating at an alarming rate, leading to a significant decline in agricultural land productivity. In addition, there are a loss of soil organic matter, nutrient depletion and loss of soil biodiversity... This may be due to urbanization, reduced breed diversity, agricultural intensification, pasture privatization and inefficient use of pesticides [1]. In order to explore and apply more effective crop quality, recent studies have been limited to soil microbes that are beneficial to plant development [2].

Mycorrhizal fungi are considered to be important microbial components in the development of major soil biogeochemical cycles (C, P, and N). Therefore, they have an important influence on the development of plants, improving their mineral and water nutrition and health. As a biofertilizer, arbuscular mycorhizal fungus (AMF) acts as

* Corresponding author: Hayet BELTAYEF

a plant growth promoter by facilitating the uptake of C, N, and P, and as a bioprotectant by protecting plants from biotic (pathogens) according to [3] and abiotic (heavy metals, high CO2 levels, and drought) stress according to [4,5]. Mycorrhizal symbioses have relationships involving an exchange of resources between the plant and the microorganisms. In this mutually beneficial combination, the AMF enhances hydromineral nutrition by including phosphate nutrition. In return, these micro-organisms receive from their host plants the products of photosynthesis necessary for their development.

Symbiosis with mycorrhizal and endophytic fungi can provide to plants with a tolerance to stress related conditions unfavorable environments.

During this work, we studied the response of the double and triple inoculation bean crop cultivation using three inoculants:

- *Rhizophagus irregularis*,

- *Serendipita indica*

-The strain CIAT 899.

2. Materials and methods

2.1. Biological and microbial materials

The plant material consists of "Contender".

Two fungal strains are used:

- ***Serendipita indica***: is an endophyte fungus obtained from the collection of cultures of the Laboratory of Ecology in Department of Plant Biology of the Faculty of Sciences of Lisbon in Portugal.
- ***Rhizophagus irregularis***: is a commercial inoculum of mycorrhizal fungi supplied by the European Bank of Glomales.

Rhizobium strain:

- ***Rhizobium* tropici** CIAT 899 is obtained from the Spanish CECT collection with the designation CECT 4654.

2.2. Growth condition

Green bean seeds are surface sterilized by soaking in solution of sodium hypochlorite for 10 minutes, then washed three times with sterile water and then germinated in pots at the rate one seedling per pot. The soil used in this experiment was taken from the experimental field of the KEF Higher Agricultural School (0-20 cm depth). Soil samples were mixed with sand (3:1) and placed in pots at a rate of 6 kg/pot to conduct this experiment.

The inoculation established with *S.indica, R. irregularis* as well as CIAT 899 is made one week after germination at the two-leaf stage. The test is established according to a random experimental Five treatments are used to study the response of bean varieties to inoculation:

2.2.1. Uninoculated plants.

- Plants inoculated with CIAT 899 and the arbuscular mycorrhizal fungus *R. irregularis.*
- Plants inoculated with CIAT 899 and the endophyte fungus *S.indica*.
- Plants inoculated with CIAT 899, *S.indica* and *R. irregularis*.

For CIAT 899 strain, the inoculation was carried out by depositing 2 mL of bacterial inoculum around the roots of each plant (about 10^9 cells/ mL).

Seedling inoculated with *S.indica* was carried out around the roots by putting 2 mL of liquid culture previously prepared in the KM medium (5.10^5 spores/mL).

For *R. irregularis*, the inoculation was carried out by layering 50 g of the commercial inoculum which is approximately 1000 spores per pot.

2.3. Agricultural parameters

2.3.1. Biomass production

At harvest (60 days of growth), aerial part and roots were carefully separated. Thus roots were washed and nodules were separated to count their number per plant. Dry biomass of shoots and roots and number of pods were determined. Three samples from each treatment were used to calculate the mean.

2.3.2. Phosphorus

Total phosphorus content was quantified colorimetrically according to the method of Murphy and Riley [6]. The absorbance was measured at 882 nm.

2.4. Root colonization by mycorrhizae fungi strains

Roots were thinned and stained with trypan blue. The mycorrhizae importance is apprehended using the frequency of mycorrhization [7].

The mycorrhizal frequency (M%) was calculated according to the following formula:

M (%) = (number of mycorrhizal root fragments/total number of roots) x 100.

2.5. Statistical Analysis

The trial model was a randomized block design (RBD). Statistical result was realized by the SAS software (1997).

3. Result

3.1. Agronomic parameters

3.1.1. Biomass yield

Plants inoculated with CIAT 899 and *S.indica* had higher dry mass of aerial and root parts than other treatments. However, despite this superiority, the difference compared to the *Rhizobium* and *R. irregularis* association is not statistically significant. Thus the control plants produce the least significant masses (Table 1). On average ($p \leq 0.001$) double infusions altered air and root dry matter. The double inoculation significantly improved ($p \leq 0.001$) the dry biomass of shoot and root.

3.1.2. Nodule number

The inoculation with CIAT 899 strain and *S.indica* increased total nodule number approximately 3 times more than non-inoculated plants. The double inoculation improved in a highly significant way ($p \leq 0.001$) the number of nodules showing the highest number compared to the other treatments (Table 1).

3.1.3. Pod number

The inoculation with CIAT 899 in association with *S.indica* or *R. irregularis* significantly improved ($p \leq 0.001$) the number of pods compared to the non-inoculated plants.

3.1.4. Mycorrhization rate

Microscopic observation of Contender variety roots is illustrated in Figure 1. The colonization is characterized by several components: Intracellular myceliums, vesicles and spores. We notice a strong colonization by *S.indica, R. irregularis* and by the association of the two strains together (Table 1). The colonization rate exceeds 70% at the pod stage. Only inoculation with fungal strains shows a highly significant effect ($p \leq 0.001$).

Table 1 Effect of *Rhizobium* and mycorrhizae fungi Inoculation on growth parameters of Contender variety

Growth Parameters	Treatments				Sig. ≤ 0.05
	Control	*R.irregularis + Ciat 899*	*S.indica + Ciat 899*	*S.indica+ R.irregularis +Ciat 899*	
Shoot dry biomass (g)	1.53 c	3.06 a	3.28 a	2.04 b	***
Root dry biomass (g)	1.19 c	2.98 a	2.99 a	2.33 b	***
Number of pods per plant	5 c	11 a	12 a	7 b	***
Nodules number per plant	96 c	273 a	295 a	185 b	***
Frequency Of Mycorhization (%)	0 b	73 a	80 a	83 a	***

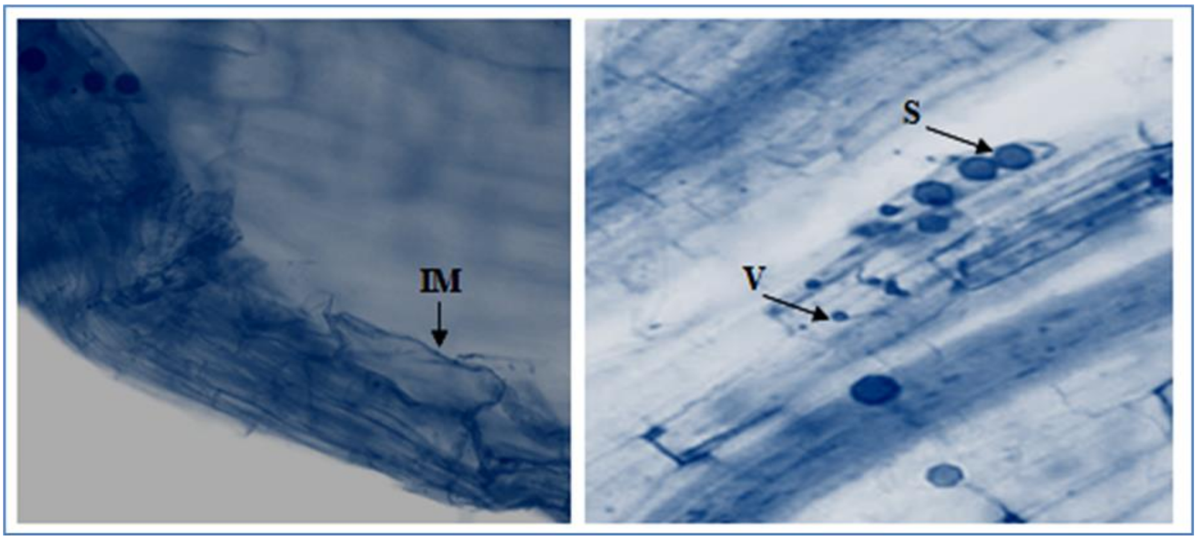

Figure 1 Contender variety roots colonized with mycorrhizae strains: *S.indica* and *R. irregularis* after 60 days of growth (Gr 40).

S: Spore; IM: Intracellular mycelium in mature root and V: vesicule

3.2. Total phosphorus concentration in shoot and root

The phosphorus contents illustrated in Figure 2 indicated that the maximum contents are obtained by the plants inoculated with *Serendipita* and CIAT 899 with an average of 1.89 ± 0.02 and 0.10 ± 0.01 mg/g DM (dry matter) in leaves and roots respectively. The double inoculation significantly improved the total phosphorus content in shoots and roots.

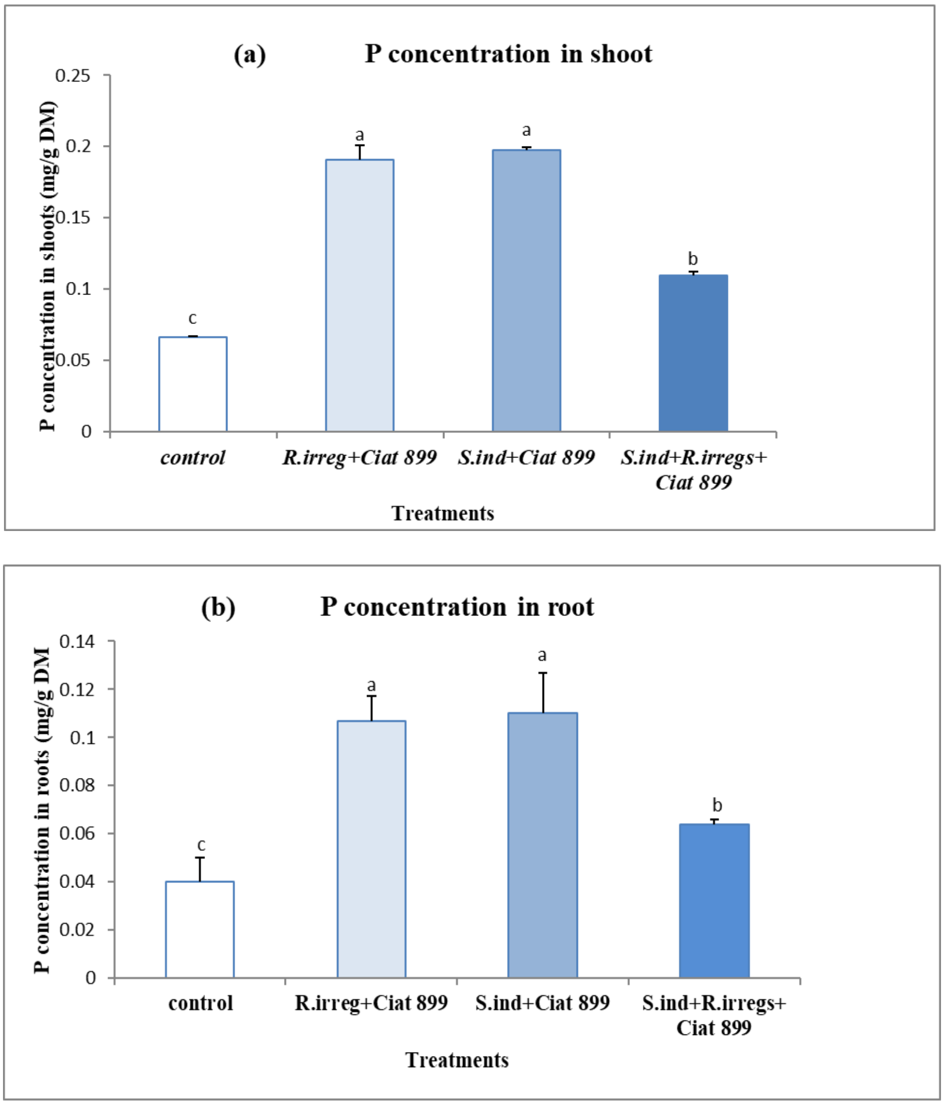

Figure 2 Phosphorus levels of Contender variety at harvest in shoot (a) and root (b)

Control: non-inoculation; *R.irreg+Ciat 899:* inoculation with *Rhizophagus irregularis* and *Rhizobium* Ciat 89; S.ind+Ciat 899: inoculation with *S.indica* and Ciat 899; S.ind+R.irregs+ Ciat 899: inoculation with *S.indica, Rhizophagus irregularis* and Ciat 899.

4. Discussion

The anthropogenic activities have contributed to the reduction of soil nitrogen and phosphorus content. In Tunisia, few researches are recognized at the impact of endophytic and mycorrhizal symbiosis on legumes cultivation. Our study is focused on the bean-mycorrhiza-endophyte interaction in case of phosphorus deficiency under controlled conditions. In fact, this work is based on the effect of *Rhizobium* Ciat 899, arbuscular mycorrhiza *R. irregularis* and the endophyte *S.indica* on the parameters of growth, yield and mineral nutrition of beans.

Our results showed that dual inoculation with *Rhizobium* Ciat 899 associated with *S.indica* or *R. irregularis* significantly improved ($p \leq 0.001$) the growth of Contender crop compared to non-inoculated plants. In particular, the inoculation with *S.indica* in association with Ciat 899 shows higher values than the other treatments for all parameters: dry biomass of shoot, dry biomass of roots and the yield of pods. These results are in agreement with the study conducted by [8] which revealed that inoculation of maize with *S.indica* showed a higher dry biomass than non-inoculated plants.

During this study, a positive interaction between common bean and *S.indica* with *R. irregularis* was confirmed. The triple inoculation increased phosphorus levels, which suggests that the competition does not affect these strains but rather we speak of a synergistic effect. These observations are in agreement with some authors who have defined that *S.indica* and *R. irregularis* lineages enhance P uptake by forming external mycelial communities in soil, thereby increasing the potential for P uptake [9]. Therefore, the development of P assimilation may also be due to capacity regulation of signals sent by plants in relation to their P deficiency. Phosphorus is stored in fungal structures in the form of polyphosphates before being transmitted to the plant at the level of the arbuscular interface [10]. Moreover, phosphorus plays an important role in various metabolic processes in plants, such as protein synthesis, photosynthesis, cell division, respiration, energy storage and nutrient transport in plants. P is an integral composant of phospholipids, nucleic acids and coenzymes activating amino acid synthesis [11].

5. Conclusion

The present study illustrates the differences between non-inoculated plants and inoculated plants with microbial strains. The inoculation with *Rhizophagus irregulis* and *Serendipita indica* associated with Ciat 899, show his potential role in promoting growth and mineral nutrition, especially phosphorus uptake of snap bean variety Contender. Further studies are needed to evaluate the performance of mycorrhizal inoculation with beans in field.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

References

[1] Merga LB, Mengistie AA, Faber JH, Brink PJ. Trends in chemical pollution and ecological status of Lake Ziway, Ethiopia: a review focussing on nutrients, metals and pesticides. Afr. J. Aquat. Sci.2020; 45:386-400.

[2] Zubek S, Błaszkowski J. Medicinal plants as hosts of arbuscular mycorrhizal fungi and dark septate endophytes. Phytochem Rev. 2009; 8:571-580.

[3] Hou L, Yu J, Zhao L and He X () Dark Septate Endophytes Improve the Growth and the Tolerance of Medicago sativa and *Ammopiptanthus mongolicus* Under Cadmium Stress. Front. Microbiol. 2020; 10:3061. doi: 10.3389/fmicb.2019.03061

[4] Santos ELD, Silva FAD, Silva FSBD. Arbuscular Mycorrhizal Fungi Increase the Phenolic Compounds Concentration in the Bark of the Stem of *Libidibia Ferrea* in Field Conditions. Open Microbiol J. 2017 ; 11:283-291.

[5] Surono, Narisawa K. The cellulolytic activity and symbiotic potential of dark septate endophytic fungus *Phialocephala fortinii* to promote non-mycorrhizal plants growth. IOP Conf. Ser.: Earth Environ. Sci. 2021; DOI 10.1088/1755-1315/648/1/012165.

[6] Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal.Chim.Acta. 1962 ; 27:31-36.

[7] Trouvelot A, Kough JL, Gianinazzi-Pearson V. Measuring the rate of mycorrhization with functional significance. 1986. Ed in Physiological and genetic aspects of mycorrhizae, Dijon, 1985, 217–21. INRA.

[8] Kumar N, , Upadhyay G, Choudhary S, Patel B, Naresh, Chhokar RS, and. Gill SC. Resource Conserving Mechanization Technologies for Dryland Agriculture. In: Naorem, A., Machiwal. Springer. 2023; 657-688, https://doi.org/10.1007/978-981-19-9159-2_33.

[9] Nadeem M, Wu J, Ghaffari H, Kedir AJ, Saleem S, Mollier A, Singh J Cheema M. Understanding the Adaptive Mechanisms of Plants to Enhance Phosphorus Use Efficiency on Podzolic Soils in Boreal Agroecosystems. Front. Plant Sci. 2022; 13:804058. doi: 10.3389/fpls.2022.804058

[10] Beltayef H, Melki M, Saidi W, Hajri R, Cruz C, Muscolo A and ben Youness M. Potential *Piriformospora indica* effect on growth and mineral nutrition of *Phaseolus vulgaris* crop under low phosphorus intake. J. Plant. Nutr.2021. 44(4): 498-507.

[11] Kayoumu M, Iqbal A, Muhammad N, Li X, Li L, Wang X, Gui H, Qi Q, Ruan S, Guo R, Zhang X, Song M, Dong Q. Phosphorus Availability Affects the Photosynthesis and Antioxidant System of Contrasting Low-P-Tolerant Cotton Genotypes. Antioxidants (Basel). 2023 Feb 12;12(2):466. doi: 10.3390/antiox12020466.