Citação:

Hayet Beltayef, Mongi Melki, Wafa Saidi, Rim Hajri, Cristina Cruz, Adele Muscolo & Mongi ben Youness (2021) Potential *Piriformospora indica* effect on growth and mineral nutrition of *Phaseolus vulgaris* crop under low phosphorus intake, *Journal of Plant Nutrition*, 44:4, 498-507, DOI: 10.1080/01904167.2020.1845366

DOI: <https://doi.org/10.1080/01904167.2020.1845366>

Potential *Piriformospora indica* effect on growth and mineral nutrition of *Phaseolus vulgaris* crop under low phosphorus intake

Hayet Beltayef, Mongi Melki, Wafa Saidi, Rim Hajri, **Cristina Cruz**, Adele Muscolo, and Mongi ben Youness

A Facult des Sciences de Bizerte, University of Carthage, Zarzouna, Tunisia;

B Ecole Suprieure d'Agriculture de kef, University of Jendouba, Tunisia;

C Regional Agricultural Development Research Center of semi-arid North-West, Kef, Tunisia;

D Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Cincias, Universidade de Lisboa, Campo Grande, Lisboa, Portugal;

E Department of Agriculture, Mediterranean University, Reggio Calabria, Italy

Abstract

The aim of this study was the mycorrhizae inoculation effect on production and mineral nutrition of snap bean variety under low phosphorus intake. The experiment was a randomized block design with four treatments: a non-mycorrhizal control, two mycorrhizal strains: *Piriformospora indica* (Piri), *Glomus intraradice* (Glomus), and their combined inoculation Piri þ Glomus; using ten replicates per treatment.

A comparative trial of different treatments on plant growth, yield parameters, mineral absorption and mycorrhizae colonization frequency was conducted in the greenhouse under controlled conditions using autoclaved soil-sand-vermiculite substrate. The inoculation with Piri and co- inoculation with PiriþGlomus significantly increased aerial dry biomass ($p < 0.01$) and highly increased root dry biomass ($p < 0.001$). Mycorrhizal snap bean plants showed a significantly higher number of pods, pods dry weight, dry weight of seeds per plant, and percentage of mycorrhizal colonization than non-mycorrhizal plants. Mycorrhizae inoculation increased significantly nitrogen and phosphorus uptake that the inoculation with Piri increased N uptake in roots by 187% and P uptake by 200% compared to the non-inoculated plants. For cations leave incorporation, Piri significantly enhanced Ca^{2+} by 100%, Mg^{2+} by 110% and NH_4^+ by 175% relative to the non-mycorrhizal plants. While mycorrhizal inoculation doesn't have a significant effect on K⁺ nutrition.

In conclusion, snap bean Contender variety seedling inoculated with Piri proved to be most effective by improving plant growth, mineral nutrition and yield production under low phosphorus uptake.

KEYWORDS: Contender snap bean variety; mineral nutrition; *Piriformospora indica*

Introduction

Mycorrhizal symbiosis is currently the subject of relevant worldwide research, both in biological sciences and agronomy fields (Wyatt et al., 2014). In Tunisia, like many developing countries, scientific progress and advances have been made toward modern agriculture with fewer inputs (chemical fertilizers, pesticide; insecticides...) to improve quality crops. In this regard, Arbuscular Mycorrhizae Fungi are known for their ability to improve plant mineral nutrition by spreading an extensive network of extra-radicular hypha that uses a greater soil beyond the rhizosphere. Mineral composition of plants is improved by the presence of AMF compared to non-mycorrhized plants, especially for less mobile elements such as phosphorus (P). Arbuscular Mycorrhizae Fungi (AMF) are also known to infect a wide range of plant species. This impressive biodiversity revealed by mycorrhizae association shows the importance of biological processes on our earth planet (Erdinc et al., 2017).

As a commercial inoculum in agriculture, *Glomus intraradice* (*Glomus*) in Glomeraceae family, is one of the best AMF strains that was extensively studied with a wide range of plants (Tian et al., 2017). The presence of *Glomus* and other endophytic fungi such as *Piriformospora indica* in the roots has many benefits for plants as a promoter microorganism.

Piriformospora indica (Piri) was discovered by Verma et al. 1998 as mycorrhizal like-fungus. Piri belonging to the family Sebacinaceae, have some equal functional aspects with AMF which allows them to have similar host plants. It is known by its power effects as bio-fertilizer, bio-regulator, phyto-mediator and plant growth stimulator for higher biomass production (Yadav et al., 2010).

A better understanding of how changes in soils affect the abundance of AMF improves their management, leading to increased productivity of poor soils and it will also be useful to define more sustainable management practices.

Tunisia has a subtropical Mediterranean climate suitable for beans crops. However, production levels are far from satisfactory which may be due to the low soil fertility and poor applied production techniques. In addition, few studies were known about the effect of mycorrhizal strains on legume cultivation in Tunisia. Anthropogenic activities have contributed to reducing phosphorus and nitrogen content in our soils. Among several approaches, a preliminary study on bean-mycorrhizal interaction has been set to solve such problems.

To improve snap bean crop yield, a study was carried out for better knowledge of the interaction between Contender variety with two mycorrhizal fungi strains (*Glomus intraradice* and *Piriformospora indica*) under low phosphorus intake in order to choose the most suitable association which affording to lead plant growth and enhance seed yield. The hypothesis was that common bean has distinct responses to inoculation with different mycorrhizae fungi species. We predict that yield, phosphorus and nitrogen uptake in mycorrhizal plants will be higher than those on non-mycorrhizae plants.

Material and methods

Microbial strains

Two mycorrhizal strains were used in these research trials: *Glomus Intraradice* was obtained from the European Bank of Glomales with the designation BEG 163. *Piriformospora indica* was obtained from Portuguese University's Center for Ecology, Evolution and Environmental Changes (CE3C).

Microbial culture conditions

The broth culture of *Piriformospora indica* was prepared in modified Hill- Kaefer medium (Kaefer, 1977). According to the optimization study of Vinod Kumar et al. (2011): 5 grown fungal solid media disks (size 5%) were placed in 500ml Erlenmeyer full by 200ml of liquid medium (20% working volume). The flask is shaken at 120 rpm in dark at 30 ± 2 C. Sporulation started on the 6th day and the inoculum was used on the 15th day in spore suspension solution

form in order to 5.10⁶ spore/ml. For *Glomus* inoculum approximately 1000 spores per 50 g of inoculum.

Co-cultivation of snap bean contender variety with Piri and *Glomus*

Seeds surface was sterilized by soaking in 3% sodium hypochlorite solution for 10 minutes and rinsed 6 times with sterile distilled water. Afterwards, seeds were sprouted in Petri dishes containing filter paper for 5 days before being sown in pots filled with 3 kg sterile substrate. The mixture substrate composed of autoclaved soil: sand: vermiculite (1:5:5) which characterized by low available phosphorus content (0.00017). Pots were placed in a greenhouse under controlled conditions with air temperature 26–35°C night-day, relative humidity 55–75% and 16h photoperiod under light intensities above 400 µmol m⁻² s⁻¹. Each pot received a single seed inoculated with mycorrhizae strain. For combined Piri+*Glomus* treatment, the plants were inoculated firstly by layering 50g of *Glomus* (Jackson et al., 1972), then seedling inoculated with Piri by soaking in spore suspension. Inoculated seedlings received once a week a modified 50% strength Hoagland solution (Heeg et al., 2008) Phosphorus-free (no P). Each pot containing one plant was watered by 100 ml distilled water daily until harvest.

Measurements

Snap bean contender plants were harvested after 60 days of growth. Each plant aerial parts, roots and pods were separated. The number of pods was determined instantly after harvest. Shoot, root, pods and grains biomass were measured after drying samples at 70°C for 72h till stable dry biomass achieved.

Root colonization by mycorrhizae fungi strains

Some Plant roots were thoroughly washed with water in a colander to remove soil particles. Roots were thinned and stained with trypan blue. Microscopic observation was done according to the lightening and staining technique of Phillips and Haymann .

The mycorrhizae importance is apprehended using the frequency of mycorrhization (Trouvelot et al., 1986). The mycorrhizal frequency (M%) was calculated according to the following

formula:

$M (\%) = \frac{1}{4} (\text{number of mycorrhizal root fragments} / \text{total number of roots}) \times 100$.

Nitrogen and phosphorus content

Plant nitrogen content in shoots and roots (% N/PI) was analyzed using the Kjeldahl method. Phosphorus content in leaves was analyzed by colorimetric assay using ammonium-molybdate-vanadate method and measured at 400 nm wavelength (Anderson [1982](#)).

Leaf cations analyses

Mineral elements (Mg^{2+} , Ca^{2+} , K^{+} and NH_4^{+}) in leaves were identified and quantified by the DIONEX ICS-1100 ion chromatography.

Statistical analysis

Trials were set into a completely randomized design. Data analysis was done using ANOVA procedure (SAS software, 1997). Significant differences were measured among treatments and analyzed by using the LSD test ($p < 0.05$).

Results

Biomass production

The dry weight of snap bean plants inoculated with Piri and Glomus was significantly higher than non-inoculated treatment ([Table 1](#)). The inoculation of plants with Piri alone or in combination with Glomus increased aerial dry biomass by 36% followed by that of Glomus alone with a 27% increase of aerial dry biomass. When the effects of different species of mycorrhizal fungi are taken into account, the biomass of plants inoculated with Piri was superior to that of Glomus. Piri Glomus increased root dry biomass by 67% as highest value. Differences in dry weight between mycorrhized and non-mycorrhized plants are significant for the above-ground biomass ($p < 0.01$) and highly significant for roots biomass ($p < 0.001$). These results agree with previous studies showing an important effect of mycorrhizae fungi to improve the growth of plants under moderate phosphorus deficiency (Faghire et al., [2010](#); Xiurong et al., [2011](#); Varma et al., [2012](#)).

Percent mycorrhizal colonization and yield production

Microscopic structures of Contender variety roots colonized with mycorrhizal strains are presented in [Figure 1](#). Colonization was characterized by the presence of numerous structures: external and internal hyphae, arbuscules, vesicles (Glomus), as well as numerous spores in roots with unequally multi-nucleated chlamydospores forms (Piri). The percent of root length colonization was significantly increased by co-inoculation with PiripGlomus compared to the treatments inoculated by one of the two strains. Mycorrhizal inoculation, regardless of the endophytic strain, enhanced yield parameter showing a higher number of pods, dry weight of pods and dry biomass of seed per plant than non-mycorrhizal plants ([Table 1](#)). The inoculation with Piri or PiripGlomus significantly increased pod and seed yields of snap bean plants ($P < 0.001$). The association of Piri and Glomus proves their potential infectivity and effectivity with Contender snap bean variety. This result was in agreement with previous research showing that mycorrhizal fungi play a crucial role in natural ecosystems by improving yield, plant growth and by inhibiting fungal phytopathogens (Habte and Osorio [2001](#); Wehner et al., [2010](#); Charoonnart et al., [2016](#)). Varma et al. ([2012](#)) revealed the potential effect of Piriformospora indica on soil bio-function benefit to plant productivity by enhancing mineral uptake.

Nitrogen content

Measured amounts of nitrogen in snap bean roots and shoots varied according to mycorrhizae strains with significant ($p < 0.001$) differences indicated in [Table 2](#). The highest value of shoot nitrogen (0.28%) was recorded under inoculated roots with PiripGlomus strains while the lowest level of 0.15% was for non-mycorrhizal plants. This result proves the beneficial functionality of mycorrhizae fungi to ameliorate nitrogen uptake. It may be due to their ability to improve soil organic matter decomposition and nitrogen uptake (Hodge et al., [2001](#)).

Phosphorus content

Phosphorus uptake by snap bean plants under inoculation with Glomus and Piri mycorrhizae strains is presented in [Figure 2](#).

Added mycorrhizae strains significantly enhanced phosphorus uptake by 1.6 fold higher than the non-inoculated control treatment. Combined inoculation with both strains of *Glomus* and Piri gave the highest significant uptake of phosphorus ($p < 0.001$).

Leaf mineral analyses and ammonium concentration

Cations concentration is illustrated in [Figure 3](#). Mycorrhizal plants showed a significant interaction with macronutrients (Mg^{2+} and Ca^{2+}) and ammonium concentration in snap bean variety. Comparing to nonmycorrhizal plants, the highest concentration of Mg^{2+} and Ca^{2+} was accumulated in plants inoculated with Piri (10.9 and 10.2 mg/g leaf dry weight respectively).

Concerning K^+ , the concentration in plants inoculated with MF was lower than nonmycorrhizal plants whatever the strain.

Discussion

In the present study, we established a symbiosis of Contender snap bean variety with different mycorrhizal inoculants. The interesting finding of this research is the significant efficiency use of nitrogen and phosphorus enhancing the growth, pod and grain yield of mycorrhizal plants under low phosphorus intake. The mycorrhizae fungi have improved plant growth under moderate phosphorus deficiency (Faghire et al., 2010 and Xiurong et al., 2011). Piri combined with *Glomus* was most effective for roots colonization, mineral uptake and yield production. This result was in agreement with previous research showing that mycorrhizal fungi play a crucial role in natural ecosystems by improving plant yield and by inhibiting fungal phytopathogens (Habte and Osorio 2001; Charoonart et al., 2016). Varma et al. (2012) revealed the potential effect of *Piriformospora indica* on soil bio-function benefit to plant productivity by enhancing mineral uptake. During testing, the inoculation with Piri and *Glomus* evinced the highest value in nitrogen absorption compared to control. The increase in nitrogen content of plants inoculated with mycorrhizal fungi may be due to their ability to improve soil organic matter decomposition and nitrogen uptake (Hodge et al., 2001). Co-inoculation of snap bean Contender variety with Piri and *Glomus* also showed the highest percent of phosphorus content in leaves which could be owing to

mycorrhizae colonization infectivity. This Improved phosphorus uptake may be due to potential signal regulation coming from plants, linked to their phosphorus deficiency. This would accord with the previous studies revealing the highest benefits of mycorrhizae fungi to plant growth under phosphorus deficiency (Tajini et al., [2012](#); Xiurong et al., [2011](#)).

The positive effect may be due to a complementarity function between mycorrhizae strains. This dual inoculation significantly improved both the phosphorus and nitrogen levels, suggesting that there was no competition between these strains. Their effects were rather synergistic (Ghaffari et al., [2016](#)) and would help ATP and phospholipids synthesis (Kristek et al., [2011](#)).

Co-inoculation with mycorrhizal strains has been debated over many years between authors who found beneficial effects and those who did not. Baxter and Dighton ([2001](#)) and Reddy and Natarajan ([1997](#)) suggested that co-inoculation with endomycorrhizal fungi strains did not necessarily make a positive effect on plant development. These authors indicated that it was difficult for mycorrhizal strains to settle in the roots cortex already colonized by native fungi or rhizobia. However, Jafari et al. ([2018](#)) reported that inoculation and co-inoculation with *Piriformospora indica* and *Glomus intraradices* improved alfalfa crop in terms of quality and yield in semi-arid field conditions. These results agree with finding of Abd El-Dayem et al. ([2015](#)) and Abeer et al. ([2016](#)) who proved that the uttermost increase in bean pod yield observed in plants inoculated with mycorrhizae fungi. Tajini et al. ([2012](#)) showed that *Rhizobium*-mycorrhizal fungus interaction or association proved that the inoculation of common beans (*Phaseolus vulgaris* L.) with the mycorrhizal fungus *Glomus intraradice* and *Rhizobium tropici* Ciat 899 significantly improved phosphorus uptake as well as symbiotic nitrogen fixation. This could be due to a great degree of specificity between Arbuscular Mycorrhizae fungi and *Rhizobium*.

In this study, we note that plants inoculated with Piri accumulated greater macronutrient (Ca^{21} , Mg^{2b}) and ammonium concentration than *Glomus*. This finding was confirmed by Durga Prasad et al. ([2019](#)) who proved the crucial effect of Piri in the enhancement of magnesium nutrition, especially under low magnesium condition. They suggested that Mg-

transporter PiMgT1 in plant roots colonized with Piri participated in magnesium uptake improving crop yield in the Mg^{2b} deficiency field.

The second messenger (Ca^{2b}) is known for its importance in symbiotic interactions especially in signal transduction. In the present work, plants inoculated with Piri showed the highest concentration in Ca^{2b} . These results were confirmed by Vadassery et al. (2009) who demonstrated that elevation of cellular Ca^{2b} content is required for the beneficial *Piriformospora indica*-*Arabidopsis thaliana* interaction for plant growth. They proved that its content was greatest in the roots.

MF, whatever the strain doesn't have a significant effect on K^b uptake showing a similar or lower concentration than non-mycorrhizal plants. This result could be explained by Liu et al. (2019) that plants grown under sufficient potassium conditions did not show a significant difference in potassium content between those inoculated with mycorrhizal and nonmycorrhizal strains. While, Ghorbani et al. (2019) proved the important effect of *Piriformospora indica* in salt tolerance in tomato seedling by enhancing K^b/Na^b homeostasis. The inoculation with *Piriformospora indica* regulated SOS1, NHXs and CNGC15 genes expression which decreased the negative effect of salt stress.

In addition, Piri also improved the ammonium concentration in the leaves. This result was confirmed by Gill et al. (2016) who showed that endophytic fungus *Piriformospora indica* improves the incorporation of ammonium into the roots.

Mycorrhizal fungi could be an appropriate application in poor soils to enhance pod and grain yield of snap bean varieties. It could decrease the exorbitant amount of chemical fertilizers used in agriculture which caused adverse effects on plant crops, agroecosystem and indirectly human health.

Conclusion

The snap bean Contender variety inoculated with Piri or co-inoculated with *Piri*/*Glomus* mycorrhizae strains revealed a significant increase in growth and yield parameters compared to non-inoculated plants. As bio-fertilizers, Piri is able to establish plant beneficial responses

by stimulating mineral nutrition of magnesium, calcium, nitrogen, phosphorus and ammonium uptake and improving production yield of snap bean variety Contender.

Further research is needed to optimizing the use of mycorrhizae inoculation with *Phaseolus vulgaris* in semi-arid regions.

References

- Abd El-Dayem, H. M., Mady, M. A., Abd El-All, M. M., and Rania, S. M. 2015. Effect of some antioxidants, potassium and arbuscular mycorrhiza on growth, yield and quality of kidney bean plants grown under water stress levels. *Annals of Agricultural Science, Moshtohor (ASSJM)* 53(1):15–30.
- Abeer, H., Alterami, S. A., Alqarawi, A. A., Alqarawi, A. A., Elsayed, A., and Egamberdieva, D. 2016. Arbuscular mycorrhizal fungi enhance basil tolerance to salt stress through improved physiological and nutritional status. *Pakistan Journal of Botany* 48(1):37–45.
- Anderson, J. P. E. 1982. Soil Respiration. In *Methods of Soil Analysis, Part 2 Chemical and Microbiological Properties. Agronomy Monograph*, eds. A. L. Page, R. H. Miller, and D. R. Keeney, vol. 9, 2nd ed., 831–71. Madison: ASA.
- Baxter, J. W., and J. Dighton. 2001. Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (*Betula populifolia*) seedlings in host–symbiont culture conditions. *New Phytologist* 152 (1):139–49.
- Charoonnart, P., K. Seraypheap, S. Chadchawan, and T. Wangsomboondee. 2016. Arbuscular mycorrhizal fungus improves the yield and quality of *Lactuca sativa* in an organic farming system. *Scienceasia* 42 (5):315–22. doi: [10.2306/scienceasia1513-1874.2016.42.315](https://doi.org/10.2306/scienceasia1513-1874.2016.42.315).
- Erdinc, C., E. D. Durak, A. Ekinçalp, S. Şensoy, and S. Demir. 2017. Variations in response of determinate common bean (*Phaseolus vulgaris* L.) genotypes to arbuscular mycorrhizal fungi (AMF) inoculation. *Turkish Journal of Agriculture and Forestry* 41:1–9.
- Faghire, M., S. Samri, A. Meddich, M. Baslam, N. Goicoechea, and A. Qaddoury. 2010. Positive effects of arbuscular mycorrhizal fungi on biomass production, nutrient status and

water relations in date palm seedlings under water deficiency. *Acta Horticulturae* 882:833–8.

Ghaffari, M. R., M. Ghabooli, B. Khatabi, M. R. Hajirezaei, P. Schweizer, and G. H. Salekdeh. 2016. Metabolic and transcriptional response of central metabolism affected by root endophytic fungus *Piriformospora indica* under salinity in barley. *Plant Molecular Biology* 90 (6):699–717. doi: [10.1007/s11103-016-0461-z](https://doi.org/10.1007/s11103-016-0461-z).

Ghorbani, A., V. O. G. Omran, S. M. Razavi, H. Pirdashti, and M. Ranjbar. 2019. *Piriformospora indica* confers salinity tolerance on tomato (*Lycopersicon esculentum* Mill.) through amelioration of nutrient accumulation, K⁺/Na⁺ homeostasis and water status. *Plant Cell Reports*, 38 (9):1151–63. doi: [10.1007/s00299-019-02434-w](https://doi.org/10.1007/s00299-019-02434-w).

Gill, S. S., R. Gill, D. K. Trivedi, N. A. Anjum, K. K. Sharma, M. W. Ansari, A. A. Ansari, A. K. Johri, R. Prasad, E. Pereira, et al. 2016. *Piriformospora indica*: Potential and Significance in Plant Stress Tolerance. *Frontiers in Microbiology* 7:332. doi: [10.3389/fmicb.2016.00332](https://doi.org/10.3389/fmicb.2016.00332).

Habte, M., and N. W. Osorio. 2001. *Arbuscular mycorrhizas: Producing and applying arbuscula mycorrhizal inoculum*, 1. Honolulu: College of Tropical Agriculture and Human Resources (CTAHR), University of Hawaii.

Heeg, C., C. Kruse, R. Jost, M. Gutensohn, T. Ruppert, M. Wirtz, and R. Hell. 2008. Analysis of the *Arabidopsis* O-acetylserine(thiol)lyase gene family demonstrates compartment-specific differences in the regulation of cysteine synthesis. *The Plant Cell* 20 (1):168–85. doi: [10.1105/tpc.107.056747](https://doi.org/10.1105/tpc.107.056747).

Hodge, A., C. D. Campbell, and A. H. Fitter. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413 (6853):297–9. doi: [10.1038/35095041](https://doi.org/10.1038/35095041).

Jackson, N. E., R. E. Franklin, and R. H. Miller. 1972. Effects of vesicular-arbuscular mycorrhizae on growth and phosphorus content of three agronomic crops. *Soil Science Society of America Journal* 36 (1):64–7. doi: [10.2136/sssaj1972.03615995003600010014x](https://doi.org/10.2136/sssaj1972.03615995003600010014x).

- Jafari, M., M. Yari, M. Ghabooli, M. Sepehri, E. Ghasemi, and A. Jonker. 2018. Inoculation and co-inoculation of alfalfa seedlings with root growth promoting microorganisms (*Piriformospora indica*, *Glomus intraradices* and *Sinorhizobium meliloti*) affect molecular structures, nutrient profiles and availability of hay for ruminants. *Animal Nutrition* 4 (1):90–9. doi: [10.1016/j.aninu.2017.08.008](https://doi.org/10.1016/j.aninu.2017.08.008).
- Kaefer, E. 1977. Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. *Advances in Genetics* 19:33–131.
- Kristek, S., A. Kristek, and H. Pavlovic. 2011. The influence of mycorrhizal fungi (*Glomus* sp.) on field pea plant survival and growth in drought caused stress conditions. *Plant, Soil and Environment* 51 (No. 9):385–9. doi: [10.17221/3601-PSE](https://doi.org/10.17221/3601-PSE).
- Kumar, V., V. Sahai, and V. S. Bisaria. 2011. High-density spore production of *Piriformospora indica*, a plant growth-promoting endophyte, by optimization of nutritional and cultural parameters. *Bioresource Technology* 102 (3):3169–75. doi: [10.1016/j.biortech.2010.10.116](https://doi.org/10.1016/j.biortech.2010.10.116).
- Liu, J., J. Liu, J. Liu, M. Cui, Y. Huang, Y. Tian, A. Chen, and G. Xu. 2019. The Potassium Transporter SIHAK10 Is Involved in Mycorrhizal Potassium Uptake. *Plant Physiology* 180 (1):465–79. doi: [10.1104/pp.18.01533](https://doi.org/10.1104/pp.18.01533).
- Prasad, D., Verma, N., Bakshi, M., Narayan, O. P., Singh, A. K., Dua, M., and Johri, A. K. 2019. Functional Characterization of a Magnesium Transporter of Root Endophytic Fungus *Piriformospora indica*. *Frontiers in Microbiology* 9:3231. doi: [10.3389/fmicb.2018.03231](https://doi.org/10.3389/fmicb.2018.03231)
- Reddy, M. S., and K. Natarajan. 1997. Coinoculation efficacy of ectomycorrhizal fungi on *Pinus patula* seedling in nursery. *Mycorrhizae* 7 (3):133–8.
- Tajini, F., M. Trabelsi, and J.-J. Drevon. 2012. Arbuscular mycorrhizas by contact with mycorrhized *Stylosanthes guianensis* enhance P use efficiency for N₂ fixation in the common bean (*Phaseolus vulgaris* L.). *African Journal of Microbiology Research* 6:1297–305.

- Tian, H., X. Yuan, J. Duan, W. Li, B. Zhai, and Y. Gao. 2017. Influence of nutrient signals and carbon allocation on the expression of phosphate and nitrogen transporter genes in winter wheat (*Triticum aestivum* L.) roots colonized by arbuscular mycorrhizal fungi. *PLoS One* 12 (2):e0172154. doi: [10.1371/journal.pone.0172154](https://doi.org/10.1371/journal.pone.0172154).
- Trouvelot, A., J. L. Kough, and V. Gianinazzi-Pearson. 1986. Measuring the rate of mycorrhization with functional significance. *Plant and Soil* 105:131–136.
- Vadassery, J., S. Ranf, C. Drzewiecki, A. Mithöfer, C. Mazars, D. Scheel, J. Lee, and R. Oelmüller. 2009. A cell wall extract from the endophytic fungus *Piriformospora indica* promotes growth of *Arabidopsis* seedlings and induces intracellular calcium elevation in roots. *The Plant Journal : For Cell and Molecular Biology* 59 (2):193–206. doi: [10.1111/j.1365-3113.2009.03867.x](https://doi.org/10.1111/j.1365-3113.2009.03867.x).
- Varma, A., A. Kharkwal, A. Agarwal, R. Bajaj, and R. Prasad. 2012. *Piriformospora indica*: The model microbe of organic green revolution. *Biofertiliser Newsletter* 1:3–8.
- Verma, S., A. Varma, K.-H. Rexer, A. Hassel, G. Kost, A. Sarbhoy, P. Bisen, B. Buetehorn, and P. Franken. 1998. *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia* 90:895–909.
- Wehner, J., Antunes, P. M., Powell, J. R., Mazukatow, J., and Rillig, M. C. 2010. Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity. *Pedobiologia* 53(2010):197–201.
- Wyatt, G. A. K., E. T. Kiers, A. Gardner, and S. A. West. 2014. A biological market analysis of the plant-mycorrhizal symbiosis. *Evolution International Journal of Organic Evolution* 68 (9):2603–18.
- Xiurong, W., P. Qiang, C. Fengxian, Y. Xiaolong, and L. Hong. 2011. Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. *Mycorrhiza* 21 (3):173–81.
- Yadav, V., M. Kumar, D. K. Deep, H. Kumar, R. Sharma, T. Tripathi, N. Tuteja, A. K. Saxena, and A. K. Johri. 2010. A phosphate transporter from the root endophytic fungus

- Piriformospora indica plays a role in the phosphate transport to the host plant. Journal of Biological Chemistry 285 (34):26532–44. doi: [10.1074/jbc.M110.111021](https://doi.org/10.1074/jbc.M110.111021).
- ance, (Edition in Physiological and genetic aspects of mycorrhizae, Dijon, 1985, 217–21. INRA
- Vadassery, J., S. Ranf, C. Drzewiecki, A. Mithofer, C. Mazars, D. Scheel, J. Lee, and R. Oelmueller. 2009. A cell wall extract from the endophytic fungus Piriformospora indica promotes growth of Arabidopsis seedlings and induces intracellular calcium elevation in roots. The Plant Journal : For Cell and Molecular Biology 59 (2):193–206. doi: [10.1111/j.1365-3113X.2009.03867.x](https://doi.org/10.1111/j.1365-3113X.2009.03867.x).
- Varma, A., A. Kharkwal, A. Agarwal, R. Bajaj, and R. Prasad. 2012. Piriformospora indica: The model microbe of organic green revolution. Biofertiliser Newsletter 1:3–8.
- Verma, S., A. Varma, K.-H. Rexer, A. Hassel, G. Kost, A. Sarbhoy, P. Bisen, B. Buetehorn, and P. Franken. 1998. Piriformoposra indica, gen. et sp. nov., a new root- colonizing fungus. Mycologia 90:895–909.
- Wehner, J., Antunes, P. M., Powell, J. R., Mazukatow, J., and Rillig, M. C. 2010. Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity. Pedobiologia 53(2010):197–201.
- Wyatt, G. A. K., E. T. Kiers, A. Gardner, and S. A. West. 2014. A biological market analysis of the plant-mycor- rhizal symbiosis. Evolution International Journal of Organic Evolution 68 (9):2603–18.
- Xiurong, W., P. Qiang, C. Fengxian, Y. Xiaolong, and L. Hong. 2011. Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. Mycorrhiza 21 (3):173–81.
- Yadav, V., M. Kumar, D. K. Deep, H. Kumar, R. Sharma, T. Tripathi, N. Tuteja, A. K. Saxena, and A. K. Johri. 2010. A phosphate transporter from the root endophytic fungus Piriformospora indica plays a role in the phosphate transport to the host plant. Journal of Biological Chemistry 285 (34):26532–44. doi: [10.1074/jbc.M110.111021](https://doi.org/10.1074/jbc.M110.111021).

Tables

Table 1. Inoculation effect of mycorrhizae fungi on shoot, root dry biomass and yield production of Contender snap bean variety.

Treatments	Shoot dry biomass (g)	Root dry biomass (g)	Number of pods/plant	Pod yield g/plant	Grain yield g/plant	Frequency of mycorrhization (%)
Control	2.22 ± 0.07c	1.04 ± 0.01c	9 ± 0.70d	1.85 ± 0.04c	2.02 ± 0.04c	0
<i>Glomus</i>	2.84 ± 0.03b	1.60 ± 0.06b	11 ± 0.57c	2.74 ± 0.06b	3.22 ± 0.02b	49.25 ± 3
<i>Piri</i>	3.047 ± 0.01a	1.64 ± 0.02b	13 ± 0.71b	2.95 ± 0.02a	3.23 ± 0.03b	60 ± 1
<i>Piri + Glomus</i>	3.042 ± 0.09a	1.75 ± 0.09a	14 ± 0.60a	2.96 ± 0.04a	3.36 ± 0.02a	65.7 ± 1
ANOVA (F-Statistic)	55.87**	127.84***	64.33***	54.97***	512.87***	184.87***

Data are means of four replications of plants harvested at 60 days of growth.

*, **, ***Significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

Table 2. Shoot and Root nitrogen content of Contender snap bean variety after 60 days growth.

	Shoot nitrogen (%)	Root nitrogen (%)
Control	0.150 ± 0.020c	0.112 ± 0.014c
<i>Glomus</i>	0.261 ± 0.010b	0.190 ± 0.012b
<i>Piri</i>	0.262 ± 0.023b	0.210 ± 0.008b
<i>Piri + Glomus</i>	0.280 ± 0.025a	0.220 ± 0.011a
ANOVA (F-Statistic)	72.73***	37.50***

Data are means of four replications of plants harvested at 60 days of growth.

***Significant at $P \leq 0.001$.

Figures

Figure 1. Snap bean root infestation with *Glomus* (1, 2) and *Piri* (3,4,5) mycorrhizae strains after 60 days of growth(Gr 40).

CS: Cluster Spore; IH: Internal Hyphae; V: Vesicle; AM: Arbuscular Mycorrhizae; S: Spore; IM: Intracellular mycelium in mature root tissue; In H: Intracellular Hyphae; EM: Extracellular Mycelium; C: Chlamydospores; MC: Multinucleate Chlamydospores.

Figure 2. Phosphorus content of Contender snap bean variety leaves after 60 days of growth. Data are means SD of four replications, significant means at $P \leq 0.001$.

Figure 3. Mineral Nutrients content (Mg^{2b} , Ca^{2b} , K^b , Na^b and NH_4^b) in the Leaves of Contender snap bean variety. Data are means SD of four replications, significant means at $P \leq 0.01$.

Fig 1

Fig 2

Figure 3