

Proceeding paper

The Effect of PGPR Inoculation on Lentil's Growth under Hydroaerobic Condition

Rim Tinhinen MAOUGAL *, Maya KECHID., Manel ABADI and Abdelhamid DJEKOUN

GBBV, freres MENTOURI University Constantine 1, 25000 Ain El Bey, Constantine, Algeria, rym.maougal@umc.edu.dz, 0021331600247

* Correspondence: e-mail@e-mail.com; Tel.: (optional; include country code; if there are multiple corresponding authors, add author initials)

† Presented at the title, place, and date.

Abstract: In Algeria, legumes are an important component of the diet because of their high protein content. They have long been considered as the most cultivated seed plants with cereals. In Algeria Lentil (*Lens culinaris*) is classified as the third legume crop after bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*). The inoculation of lentils with Plant Growth-Promoting Rhizobacteria (PGPR) has gained significant attention in agricultural research due to its potential in enhancing plant growth and improving crop productivity. 14 PGPR isolated from the region of Constantine, Algeria were investigated for their ability to promote the cultivation of this legume under hydroponic conditions. The isolated strains had a stimulatory effect on growth. In hydroaerobic conditions application of PGPR significantly increased shoot length, root length, fresh weight and dry weight by 178 %, 169 %, 350 % and 311 % as compared with the uninoculated control. PGPR also show an excellent accumulation of phosphorus in the root part compared to the aerial part, specifically for bacteria 29. These indicated that the isolated PGPR strains can be utilized as potential biofertilizers for stimulating growth of lentil

Keywords: PGPR; (*lens culinaris*); Inoculation; hydroaerobic; phosphorus uptake

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1. Introduction

Researchers and agriculturists are always investigating novel approaches to enhance crop productivity and resilience in the aim of sustainable agriculture and increased food security [1]; utilizing plant growth promoting rhizobacteria (PGPR) has emerged as one of these methods that shows promise for boosting the development and production of a variety of crops [2] such as lentils (*Lens culinaris*)

Due to their high protein content, lentils are a significant leguminous crop that significantly contributes to the food and nutritional security of low-income families [3] however, a variety of element, such as soil health, environmental stresses and microbial interaction, might affect how they are produced [4]. A type of beneficial bacteria known as PGPR, which live in the rhizosphere, have drawn attention for their capacity to form symbiotic relation with plants, promoting nutrient uptake, enhance growth and give resistance against pathogens [1, 5].

Inoculation of lentils with PGPR agents has been shown to improve plant growth, nutrient mobilization, and yields [6] this suggests that inoculation of lentils with PGPR could be a promising approach. The aim of the study was to assess the possible use as inoculant, bacteria isolated from rhizosphere lentils to evaluate the accumulation of P and increasing the productivity by minimizing the fertilization.

2. Materials and Methods

The experimental design consisted of randomized complete block with 3 replications and 2 treatments: lentils with PGPR inoculation and Lentils without PGPR inoculation (control)

2.1. Plant material and inoculation

The lentil (*Lens culinaris*) genotype used in this study is syrie 229 variety, a local selection on an introduced population of Syria [7]. Seeds were surface-sterilized by immersing them in 70% ethanol for 5 minutes, followed by a 1.5% calcium hypochloride solution for 10 minutes, and then thoroughly washed with sterile distilled water. They were germinated for 3 days at 28°C in the dark.

A well characterized and efficient PGPR strain suitable for lentil growth promotion was selected based on previous studies [8]. The selected PGPR strain was cultured on LB medium and incubated at the optimal temperature for growth. The inoculation was performed by soaking the seeds of lentils for 30 min within a freshly prepared suspension of bacteria containing 10^8 bacteria ml⁻¹.

2.2. hydroponic system experiments

Lentil seeds were sown in the hydroponic containers consisting of a container with nutrient solution [9]. prepared according to the recommended concentrations for lentil growth. The solution contained 2 mm urea as starter N during the first 15 days of transplantation. Once the radicle has reached 5 cm the germinated seeds were inoculated and transferred into 30 l wats containing 10 plant per vat, the PGPR inoculum was introduced into the nutrient solution, maintaining a final concentration as per the experimental design. Control containers received sterile nutrient broth

2.3. Growth parameters

The plants were harvested after 6 weeks of growth, length and fresh weight of shoot and roots plants were measured. Lentil shoot, roots were separated and dried at 70° C for 48 h and shoot and roots dry weight were measured.

2.4. phosphate measurement

Total P of plants was determined calorimetrically uses the molybdophosphate method (Murphy and Riley) after digestion with nitric-perchloric acids (6:1, V:V) at 250 °C for 6h.

2.5. statistical analysis:

Data obtained were subjected to analysis of variance (ANOVA) using Excel stat software version 2009 to determine significant differences between treatments.

3. Results

3.1. Effet of PGPR on lentils growth

Inoculation on lentils with different PGPR isolates had a positive effect on the growth compared with the control (Table 1). 6 weeks after inoculation, we observed that the 9 bacteria and the OL 13 control showed significantly different results (P 0.05) (Table 1).

Table 1. Effects of bacteria isolated from *Lens culinaris* rhizosphere on *lens culinaris* shoot, root growth 6 weekds after inoculation.

Bacterial strains	Shoot length (cm)	Root length (cm)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
<i>OL13</i>	17.45±1.86	22.43±1.35	24.28±2.03	19.04±0.02	47.14±3.23	6.71±0.06
<i>Bac 24</i>	18.38±1.84	24.47±3.44	178.57±4.61	50.77±2.41	70.01±2.06	23.84±1.90

<i>Bac 58</i>	28.83±1.86	23.55±2.85	277±1.09	56.71±13.9	147.14±1.31	31.85±1.94
<i>Bac 82</i>	15.02±2.39	38.53±1.11	307±1.41	56.28±1.7	75.71±1.36	25.57±2.34
<i>Bac 23</i>	20.17±1.84	26.37±4.87	260±1.03	51.71±2.92	211.42±2.4	35.57±1.65
<i>Bac 6</i>	15.45±1.86	26.47±3.54	186.85±1.17	46.42±1.26	102.85±3.5	36.42±1.94
<i>Bac 49</i>	18.03±2.39	21.8±1.5	123.14±2.81	48.42±1.91	151.42±1.7	25.57±1.92
<i>Bac 29</i>	28.55±1.84	35.5±4.26	119.57±2.53	38±2.43	134.28±2.0	22.14±2.71
<i>Bac 17</i>	21.26±1.86	24.76±2.44	128.57±1.92	58.57±3.08	190±2.4	30.57±1.49
<i>Bac 46</i>	17.17±2.39	24.3±12.06	127.66±2.65	55.66±2.17	251.66±3.6	27.33±1.25

Six of the 9 bacteria showed particularly high growth promotion ability: 24, 58, 82, 23, 6, 29, 46 these bacteria induced a high increase in fresh weight of shoot up to 13 times higher than that the control. Their promoting effect was also noted in growth of root, their fresh weight was almost 5 times higher that of the control for the isolate 46. This promotional impact was confirmed by data on dry weight of shoot and roots.

In terms of root length, the 9 bacteria PGPR inoculum showed significantly different results (Table 1) the isolate 82 displayed the highest root length at 38.54 cm and the isolate 49 showed the lowest root length with averages of 21 cm.

The PGPR isolates increased the shoot length of lentils compared to the control and the bacteria's 58 and 29 was the most effective promoter of shoot length.

3.2 Phosphorus content on lentils

Phosphorus content of the shoot lentils was affected by the PGPR inoculation (Figure 1), bacterial isolates significantly increased the P contents both in roots and shoot compared to the control up to 150%. The isolate 29 and the isolate 46 performed significantly better than the others on shoot and roots respectively. Overall, the bacterial effect was more pronounced on root than shoot.

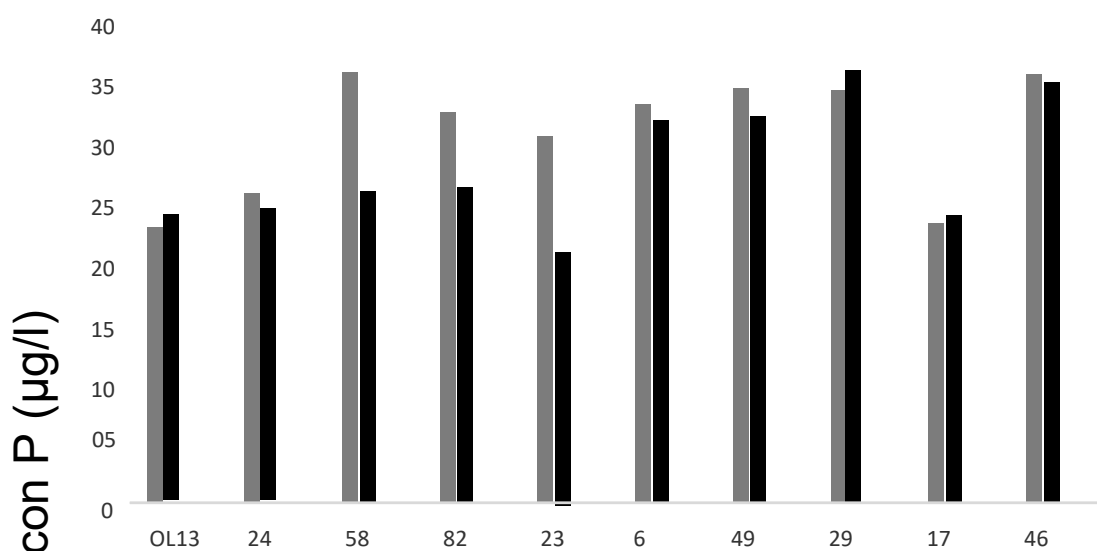


Figure 1. Effect on inoculation on concentration of Phosphorus (P) on roots (grey bars) and shoots (black bars) of *Lens culinaris* inoculated with PGPR grown in hydroponic culture harvested at 6 weeks after sowing.

4. Discussion

The benefit effect of PGPR on plant growth have been demonstrated by a variety of research [10, 11, 5, 12]. In this study we inoculated bacterial strains already isolated from

lentils rhizosphere. The current research demonstrated that all bacteria isolated from lentils rhizosphere could be effective in enhancing the *lens culinaris* growth under hydroponic condition.

Although variation between different bacterial strains were small, all bacterial inoculation caused significant differences on the plant parameters compared to the control. A positive effect on shoot and root development was observed. This result has also been reported by many other works [13, 14]. Six out of the nine bacteria examined proved to be most effective promoters of the lentils shoot and root development

Inoculation of lentils resulted in an increase content of Phosphorus as compared to the control. Generally, solubilization of insoluble compound including P is due to the excretion of microbial metabolites as organic acids [15] or enzymes as phosphatases and phytases [16, 17]. studies showed that a higher nutrient uptake by inoculated roots significantly improved plants growth, so PGPR inoculations may increase the surface area and the length of roots [18].

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References

- Balázs, E.; Horn, P.; LHornok, L.; Kovács, M.; Rajkai, K.; Szendrő, Z. Reflections on the Regenerative Agriculture Report: Bioeconomical balances and the potential of biotechnology, EFB Bioeconomy Journal, **2023**, Volume 3,
- Backer, R.; Rokem, J.S.; Ilangumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Subramanian, S.; Smith, D.L Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Front. Plant Sci.* **2018**, *9*:1473. doi: 10.3389/fpls.2018.01473
- Tena, W.; Wolde-Meskel, E.; Walley, F. Symbiotic Efficiency of Native and Exotic *Rhizobium* Strains Nodulating Lentil (*Lens culinaris* Medik.) in Soils of Southern Ethiopia. *Agronomy* **2016**, *6*, 11. <https://doi.org/10.3390/agronomy6010011>
- Wu, D.; Wang, W.; Yao, W.; Li, H.; Wang, Q.; Niu, B. Microbial interactions within beneficial consortia promote soil health. *Science of The Total Environment*, **2023**. Volume 900,
- Vacheron, J.; Desbrosses, G.; Bouffaud, M.L.; Touraine, B.; Moëgne-Loccoz, Y.; Muller, D.; Legendre, L.; Wisniewski-Dyé, F.; Prigent-Combaret, C. Plant growth-promoting rhizobacteria and root system functioning. *Front. Plant Sci.* **2013**, *4*:356. doi: 10.3389/fpls.2013.00356
- Babu, S.; Prasanna, R.; Bidyarani, N.; Nain, L.; Singh, Y. S. Synergistic action of PGP agents and *Rhizobium* spp. for improved plant growth, nutrient mobilization and yields in different leguminous crops. *Biocatalysis and Agricultural Biotechnology*, **2015**, Volume 4, Issue 4 : Pages 456-464,
- <https://www.itgc.dz/wp-content/uploads/2019/07/LA-LENTILLE.pdf> (accessed on 12 oct 2023)
- Laadjabi, C. Isolement et identification des bacteries dans la rhizosphere de 5 varietes de feves et determination de leurs caracteres PGPR, master, université freres Mentouri Constantine, Algeria. 17/7/2019.
- Hernandez, G.; Drevon, J.J. *In situ* assay of acetylene reduction by *Phaseolus vulgaris* root nodules: influence of oxygen. *Journal of Plant Physiology*, **1991**, *138*:587-591.
- Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbiol Biotechnol.* **2012**, *28*:1327–1350
- Glick, B.R. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*. **2012**, doi:10.6064/2012/963401
- Kisiel, A.; Kepczyńska, E. *Medicago truncatula* Gaertn. as a model for understanding the mechanism of growth promotion by bacteria from rhizosphere and nodules of alfalfa. *Planta* **2016**, *243*, 1169–1189 <https://doi.org/10.1007/s00425-016-2469-7>
- Glick, B.R. The enhancement of plant growth by free-living bacteria. *Can J Microb.* **1995**, *41*:109–117
- Martinez-Viveros, O.; Jourquera, M.A.; Crowley, D.E.; Gajardo, G.; Mora, M.L. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J Soil Sci Plant Nutr*, **2010**, *10*:293–319.
- Roriguez, H.; Gonzalez, T.; Goire, I.; Bashan, Y. Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. *Naturwissenschaften*, **2004**, *91*:552–555
- Maougal, R. T.; Brauman, Alain.; Plassard, C.; Abadie, J.; Djekoun, A.; Drevon, J. J. Bacterial capacities to mineralize phytate increase in the rhizosphere of nodulated common bean (*Phaseolus vulgaris*) under P deficiency. *European Journal of Soil Biology*, **2014**, *62*, p. 8-14. doi.org/10.1016/j.ejsobi.2014.02.006

17. Maougal, R.T., Bargaz, A. ; Sahel, C. ; Amenc, L. ; Djekoun, A. ; Plassard, C. ; Drevon, J.J. Localization of the *Bacillus subtilis* beta-propeller phytase transcripts in nodulated roots of *Phaseolus vulgaris* supplied with phytate. *Planta*. **2014**;239(4):901-8. doi: 10.1007/s00425-013-2023-9.
18. Bashan, Y.; Holguin, G.; de-Bashan, LE. Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances. *Can J Microbiol* , **2004**, 50:521–577

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