Assessment of Arbuscular Mycorrhizal Fungi Status and Rhizobium on Date Palm (*Phoenix dactylifera* L.) Cultivated in a Pb Contaminated Soil

Mouloud Ghadbane, Samir Medjekal, Laid Benderradji, Hani Belhadj, and Harzallah Daoud

Abstract Rhizobia and mycorrhizal fungi promote non-Legumes growth. This study investigated the ability of *Rhizobium leguminosarum* GM01 and *Glomus* spp. A01 isolates on the growth and uptake of lead (Pb) by date palm (*Phoenix dactylifera* L.) grown in heavy metal-polluted soils. The combination of A01 with GM01 increases the mycorrhizal colonization of date palm by arbuscular mycorrhizal fungi (AMF) with frequency (*F%*) of 86.67%. The selected isolates GM01 and A01 increased all plant growth parameters of date palm. The initial results indicate that the AMF and *Rhizobium* as a promising agent for promoting growth of plants. The metal tolerance trait exhibited by A01 and GM01 strains indicates their potentials as effective agents for phytoremediation of heavy metals in polluted environments. This ability may be of particular importance during restoration practices into habitats with a history of heavy metal pollution.

Keywords Plant growth · Heavy metal · Phytoremediation · *Glomus* · *Rhizobium*

1 Introduction

Heavy metal toxicity has become a major limiting factor for the global agricultural production. Soil contamination by heavy metals results from various sources, such as atmospheric deposition, the use of sewage sludge in agricultural land, and the

M. Ghadbane (✉) · L. Benderradji
Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila, M'sila, Algeria
e-mail: mouloud.ghadbane@univ-msila.dz

S. Medjekal
Department of Microbiology and Biochemistry, Faculty of Science, University Mohamed Boudiaf of M'sila, M'sila, Algeria

M. Ghadbane · H. Belhadj · H. Daoud
Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University Ferhat Abbas Setif 1, 19000 Setif, Algeria

M. Ksibi et al. (eds.), *Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions (2nd Edition)*, Environmental Science and Engineering, https://doi.org/10.1007/978-3-030-51210-1_111

exploitation of mineral resources [1]. Heavy metal removal from soils and water can be achieved by a sand rich in iron oxide [2], microorganisms [3], microalgae [4], and enzymes [5]. Benefits of heavy metal tolerant-plant growth-promoting microorganisms are immense as they perform multiple functions such as improved soil quality, enhanced plant growth, detoxification, and removal of heavy metals from soils [6]. Bioremediation of contaminated sites has been allocated much attention globally as a procedure to remediate soil quality and eliminate metals from soils. Phytoremediation is an established approach to remove contaminants from the environment by plants. But using plants alone for bioremediation faces many limitations. If these metal accumulating plants are assisted by metal-resistant growth promoting bacteria and AMF, the efficiency is increased several folds. This strategy of phytoremediation with appropriate heavy-metal-resistant rhizobacteria is gaining more attention worldwide. *Rhizobium* strains colonize the roots of tomato and pepper plants promoting their growth in different production stages increasing yield and quality of seedlings and fruits [7]. The ability of rhizobia to promote the growth of cereals such as maize, barley, and rice is well known. The objective of the present study was to study the influence of *Glomus* spp. A01 and *Rhizobium leguminosarum* GM01 inoculation on the growth of date palm (*Phoenix dactylifera* L.) exposed to Pb.

2 Materials and Methods

2.1 Sampling and Cultivation of Rhizobium and AMF Species

Rhizobium leguminosarum GM01 and *Glomus* spp. A01 were isolated from *Calobota saharae* and *Astragalus gombo* Coss. & Dur. and date palm (*Phoenix dactylifera* L.), respectively, growing in the desert region Biskra, Algeria (Fig. 1).

2.2 Metal Treatments and Determination of AMF Root Colonization

The biocontrol efficacy of *R. leguminosarum* GM01 and *Glomus* spp. A01 isolates were studied in growth room conditions. The pot-culture experiment was arranged in randomized design containing four treatments: plant without AMF and *Rhizobium* inoculation (Control), plant inoculated with *Glomus* spp. A01, plant inoculated with Rhizobium, plant co-inoculated with *Glomus* spp. and *R. leguminosarum* GM01 exposed to Pb contaminated soil with an initial concentration of $Pb(NO_3)_2$ 200 mg/L. The germination index and growth parameters were determined after six months. AMF root colonization was estimated by light microscopy as described by Trouvelot et al. [8]. The experiment was conducted with three replicates per treatment.

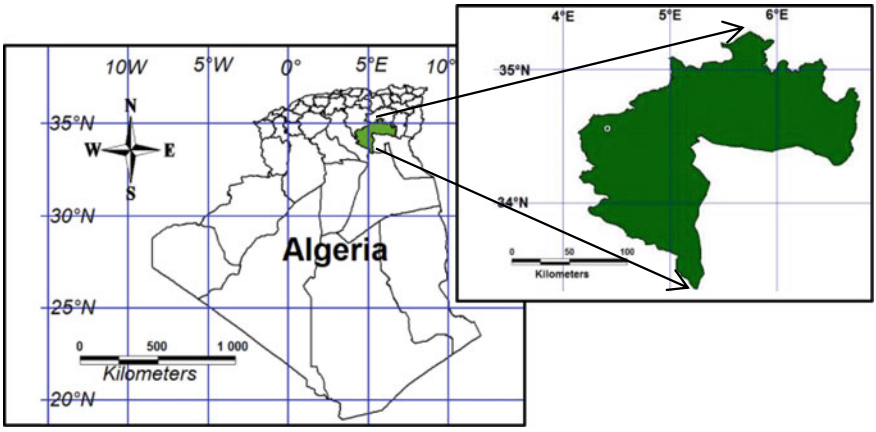

Fig. 1 Sites from which the rhizosphere soil samples were collected

2.3 Statistical Analyses

All results were analyzed statistically with SAS software 9. The experiment was established under a completely random design with four inoculation treatments and three replicates each. Statistical differences between means were determined by one-way ANOVA with Duncan's multiple range with a level of significance established at $P < 0.05$.

2.4 Results

The results showed that the date palm (*Phoenix dactylifera* L.) successfully infected the roots indicated by the AM fungi structures. It can be confirmed that all date palms were colonized by AMF in all treatments because at least one of the typical structures of vesicles or arbuscules was found in the root. Furthermore, some other structures, such as intra and intercellular hyphae, and hyphal coils of AMF were abundant in most of plant roots and sometimes the intraradical spores could be observed. Mycorrhizal colonization frequency (*F%*) of date palm by AMF ranged from 52.80 to 86.67% (Fig. 2).

The highest colonization with AMF of date palms was with A01 and GM01co-inoculation (Fig. 2). The highest root length, root fresh weight, shoot height, shoot fresh weight, and germination index of date palm (*Phoenix dactylifera* L.) occurred in plants co-inoculated by A01 and GM01, which differed significantly from the control or other treatments (Table 1). Mycorrhizal plants showed longer seedling, wider basal diameter, higher dry biomass, and root/shoot ratio compared with non-mycorrhizal plants (Table 1).

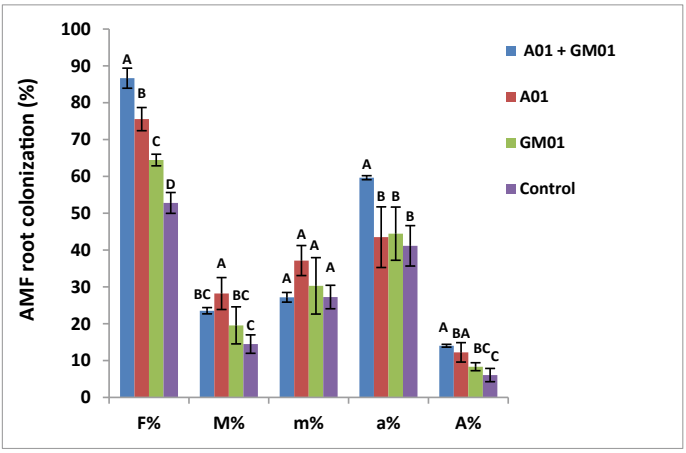

Fig. 2 Effect of *Glomus* spp. A01 and *Rhizobium leguminosarum* GM01 strains on mycorrhizal colonization of date palm (*Phoenix dactylifera* L.) treated with Pb; (F %) mycorrhizal colonization frequency, (M %) relative mycorrhizal root length, (m %) intensity of colonization within individual mycorrhizal roots, (A %) relative arbuscular richness, and (a %) arbuscule richness in root. Control: Plant without AMF and *Rhizobium* inoculation

Table 1 Effect of *Glomus* spp. A01 and *Rhizobium leguminosarum GM01* strains on the root length, root fresh weight, shoot height, shoot fresh weight, and germination index of date palms (*Phoenix dactylifera* L.) treated with Pb

Strains	Root length (cm)	Root fresh weight (g)	Shoot height (cm)	Shoot fresh weight (g)	Germination index (%)
A01	34.67 ± 2.7b	4.17 ± 0.7a	28.83 ± 0.9b	5.5 ± 0.4a	82.0 ± 2.4bac
GM01	25.33 ± 1.4c	2.37 ± 0.1b	24.33 ± 1.4c	3.4 ± 0.5b	85.33 ± 2.7b
GM01 + A01	46.0 ± 3.6a	4.6 ± 0.4a	35.67 ± 2.3a	5.83 ± 0.3a	92.33 ± 1.9a
Control	22.5 ± 1.3c	2.5 ± 0.2b	17.17 ± 0.8d	2.1 ± 0.1c	77.67 ± 2.3c

The values presented in the table are means ± standard errors. The same letter in the row indicates not significantly different values. Control: plants without A01 and GM01 strains

3 Discussion

Beneficial rhizobia can directly influence the physiology of the plants and in addition to directly interacting to beneficially influence the mycorrhizal relationship and/or plant growth. Rhizobia together with AMF have been studied to create a more indirect synergism that supports plant growth including nutrient acquisition, inhibition of plant pathogenic fungi, and enhancement of root branching [9]. The results confirmed the potential of applying mycorrhizal and PGPR biotechnology in sustainable date palm culture in Pb contaminated soil. Rhizobia can accumulate,

transform, or detoxify Pb. In general, the benefit from these microbes can have a vast impact on plant's health.

4 Conclusion

The Pb stress conditions influence date palm growth, resulting in the decline of root and shoot vigor. Inoculation with A01 alone or in combination with GM01 increased successfully the mycorrhizal colonization and can significantly increase the root vigor and biomass accumulation of date palms in Pb contaminated soil. Therefore, inoculating date palm with A01 and GM01 can promote the host tolerance to Pb by reducing the toxicity of Pb to date palms. The findings proved the success of A01 and GM01 as an effective inoculum in soil polluted with Pb and definite potential in phytoremediation of Pb contaminated soil. This biotechnology approach would help rehabilitate contaminated soils.

References

1. Lin, R., Wang, X., Luo, Y., Du, W., Guo, H., Yin, D.: Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). Chemosphere **69**, 89–98 (2007)
2. El Haouti, R., Anfar, Z., Et Taleb, S., Benafqir, M., Lhanaf, S., El Alem, N.: Removal of heavy metals and organic pollutants by a sand rich in iron oxide. Euro-Mediterranean J. Environ. Integr. **3**, 17 (2018)
3. Dutta, P., Karmakar, A., Majumdar, S., Roy, S.: *Klebsiella pneumoniae* (HR1) assisted alleviation of Cd(II) toxicity in *Vigna mungo*: a case study of biosorption of heavy metal by an endophytic bacterium coupled with plant growth promotion. Euro-Mediterranean J. Environ. Integr. **3**, 27 (2018)
4. Das, D., Chakraborty, S., Bhattacharjee, C., Chowdhury, R.: Biosorption of lead ions (Pb^{2+}) from simulated wastewater using residual biomass of microalgae. Desalin. Water Treat. **57**, 4576–4586 (2016)
5. Nath, A., Chakraborty, S., Bhattacharjee, C.: Bio-reactorand enzymatic reactions in bioremediation. In: Das, S. (ed.) Microbial Biodegradation and Bioremediation, pp. 455–495. Elsevier, Amsterdam, Netherlands (2014)
6. Mishra, J., Singh, R., Arora, N.K.: Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Front. Microbiol. **8**, 1706 (2017)
7. García-Fraile, P., Carro, L., Robledo, M., Ramírez-Bahena, M.H., Flores-Félix, J.D., et al.: Rhizobium promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS ONE **7**(5), e38122 (2012)
8. Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V.: Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson, V., Gianinazzi, S. (eds.) Physiological and Genetical Aspects of Mycorrhiza, pp. 217–221. INRA Presse, Paris (1986)
9. Patil, G.B., Lakshman, H.C., Mirdhe, R.M., Agadi, B.S.: Effect of co-inoculation of AM fungi and two beneficial microorganisms on growth and nutrient uptake of *Eleusine coracana* Gaertn. (Finger millet). Asian J. Plant Sci. Res. **3**, 26–30 (2013)