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Science for resilient livelihoods in dry areas

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Exploring the Plant Growth Promotion potential of Rhizobium under abiotic stress

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Introduction

In the arid and semiarid regions, crops are seriously affected by many abiotic stresses. Drought and salinity are the main abiotic stresses that impact negatively plants growth by reducing directly the plants yields through disrupting the biochemical, physiological and genetic homeostasis within plant cells (Kavamura et al., 2013). In fact, both stresses impact negatively in the synthesis of plant growth regulators such as auxin, gibberellin and cytokinin and trigger the production of stress hormones like abscisic acid (ABA) and ethylene, which inhibit plant growth through several mechanisms (Yang et al., 2009).Plant-associated microbes, such as **Rhizobia**, play a vital role in plant growth under abiotic stress by modifying the root system, enhancing mobilization and the uptake of several essential elements, and modulating physiological parameters (Egamberdieva et al., 2018).

In this study, we assessed some Plant Growth Promotion (PGP) traits of a group of Rhizobium strains isolated from root nodules of lentil, their growth tolerance under drought and salt conditions and their PGP activity under these abiotic conditions.

Material and methods

- **Measurement of phosphate solubilization activity and IAA production**

Strains were qualitatively tested for their ability to solubilize Phosphate using Pikovskaya's agar (Paul & Rao, 1971) Phosphate solubilization efficiency was calculated using the following formula $S.E\,\% = \frac{Z-C}{C} \times 100$. Z: solubilization zone (mm), and C: colony diameter (mm). The measurement of phosphate solubilization quantity of the strains was carried out by using the protocol of Kothamasi and al., (2006) with some modifications. the absorbance of the solution was read at 430 nm in a spectrophotometer. IAA production was determined by quantifying the indolic compounds using Gordon and Weber method (1951). The pink-auxin complex was read at 530nm in a spectrophotometer.

- **Assessment of bacterial growth, Phosphate solubilization activity and IAA (Indol Acetic Acid) production under abiotic stress**

1% culture of the strains (cell≈10^8 CFU/ml) were grown in modified Medium BIII, Pikovskaya broth medium and YAM + L-tryptophan (5mM) broth medium, with different osmotic potentials (ψ= -0,53; ψ=-0,75; ψ=-1,203; ψ=-1,77; ψ=-2,6; ψ=-3,7 MPa), prepared by using PEG (Michel and Merrill, 1973) and different concentrations of NaCl (0.5%, 1%; 1.5%; 2%; 3%; 4%; 5%).

- **Statistical analysis and Graphs Drawing**

Data analysis was carried out by Excel 2013 (Microsoft, Redmond, USA) and SPSS 20 (IBM Corp., Chicago, USA) using test of Homogeneity of variances, robust tests of equality of means (Welch`s test and Brown-Forsythe`s test) and One-way analysis of variance with at the least significant difference (LSD) analysis (P≤0.05).

Results

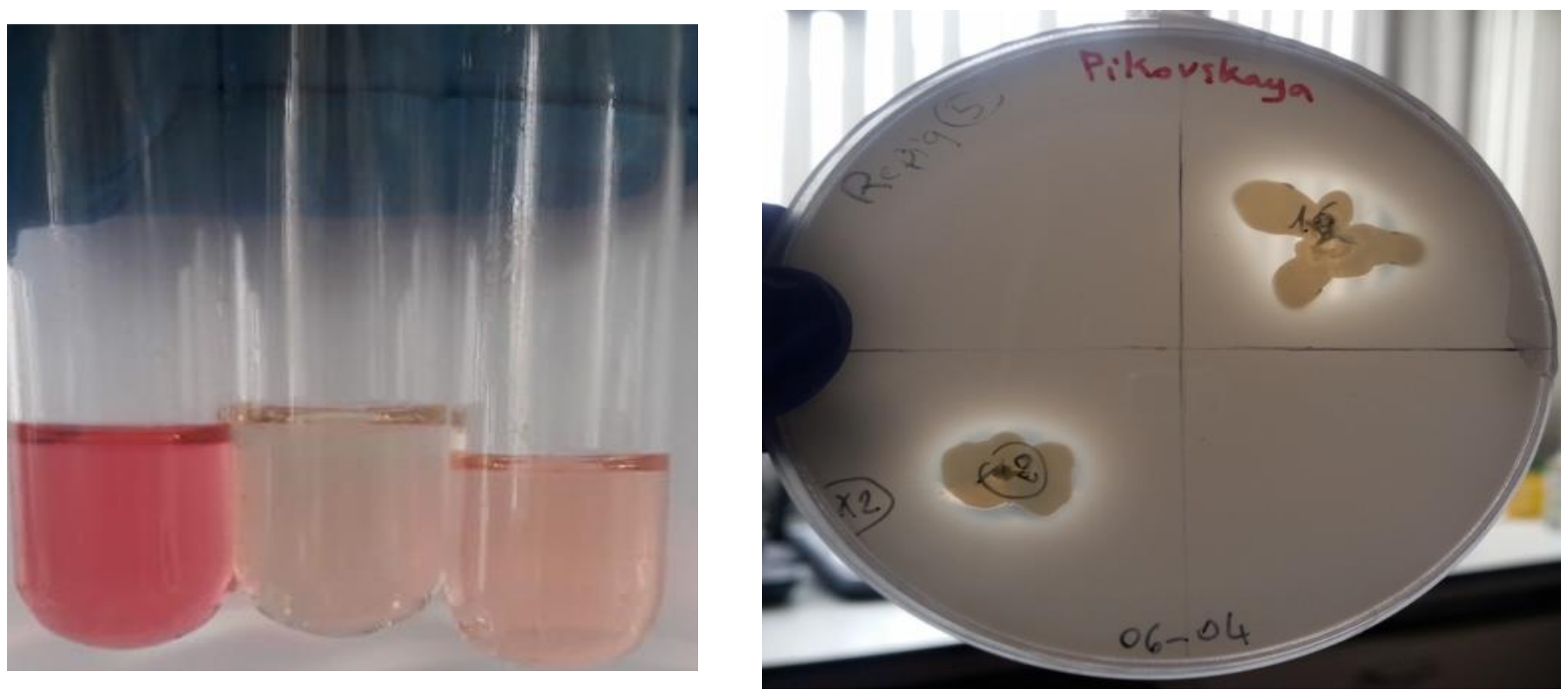

Figure 1 –the development of The pink-auxin complex in the bacterial supernatant after adding the Salkowski`s reagent (on the left).the isolated Rhizobium strains 1159N24 and 512N1(Image on the right).

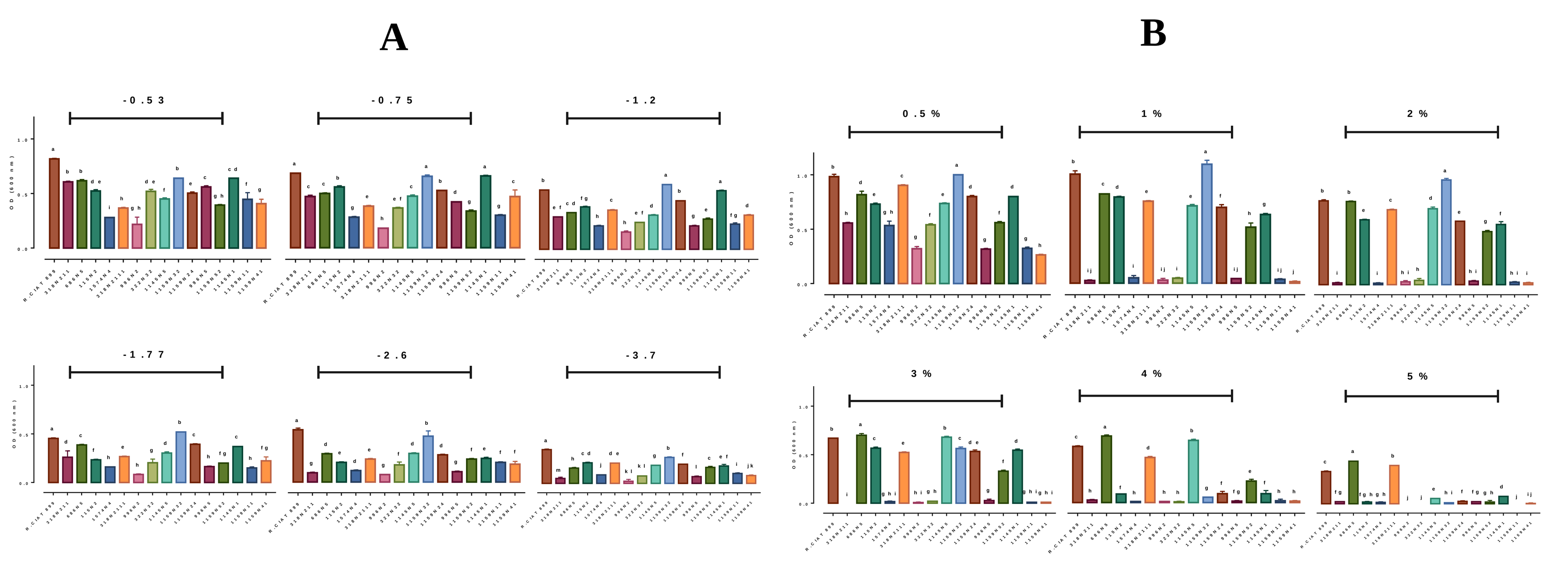

Figure 3 – Bacterial growth under drought stress (A) and salt stress (B). Different letters represent significant statistical differences by using the least significant difference (LSD) (P < 0.05). O.D : Optical Density.

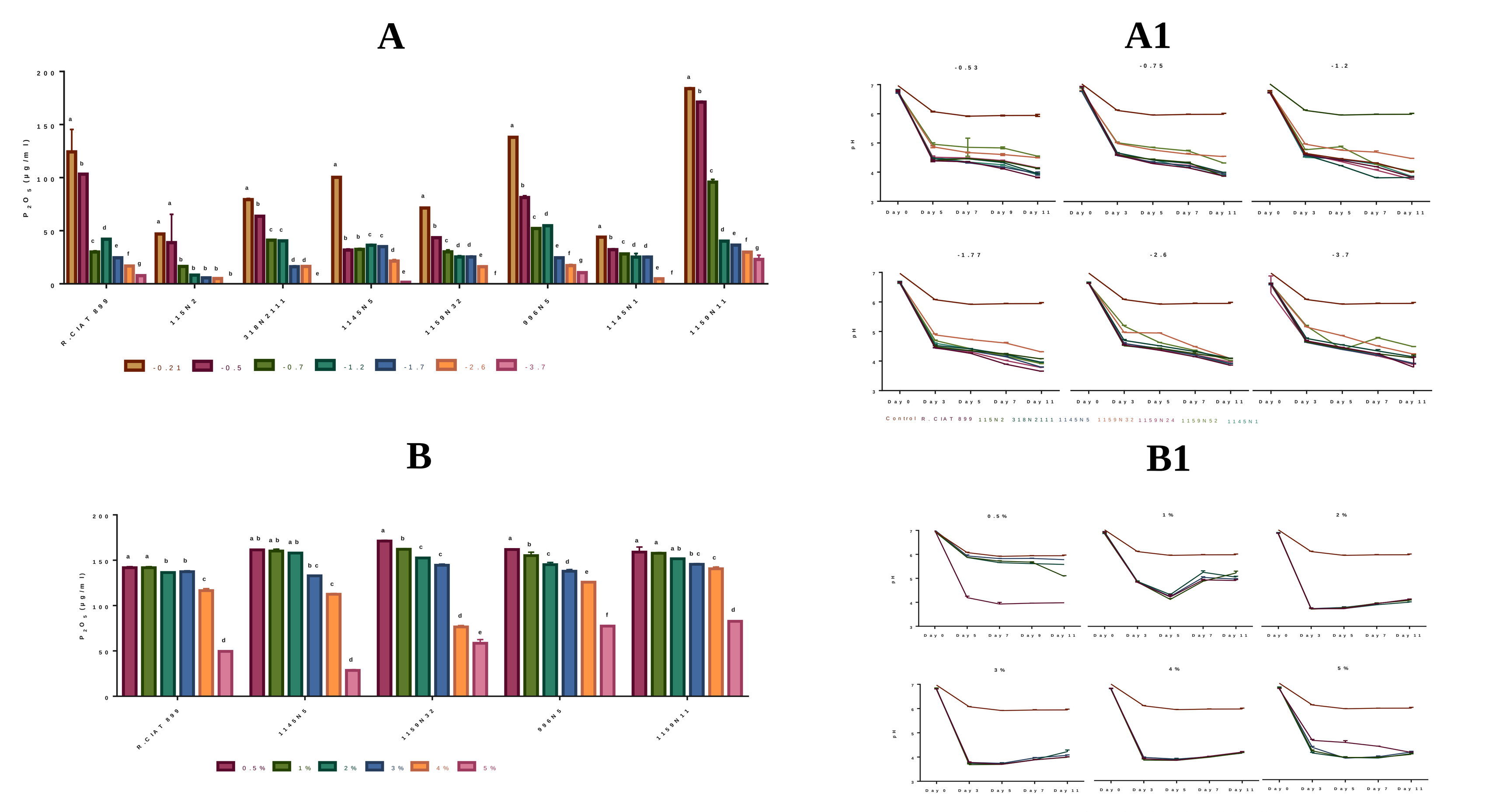

Figure 4 – Phosphate solubilization activity (ug/ml) and pH deviation under Drought stress(A and A1) and salt stress(B and B1). Different letters represent significant statistical differences by using the least significant difference (LSD) (P < 0.05).

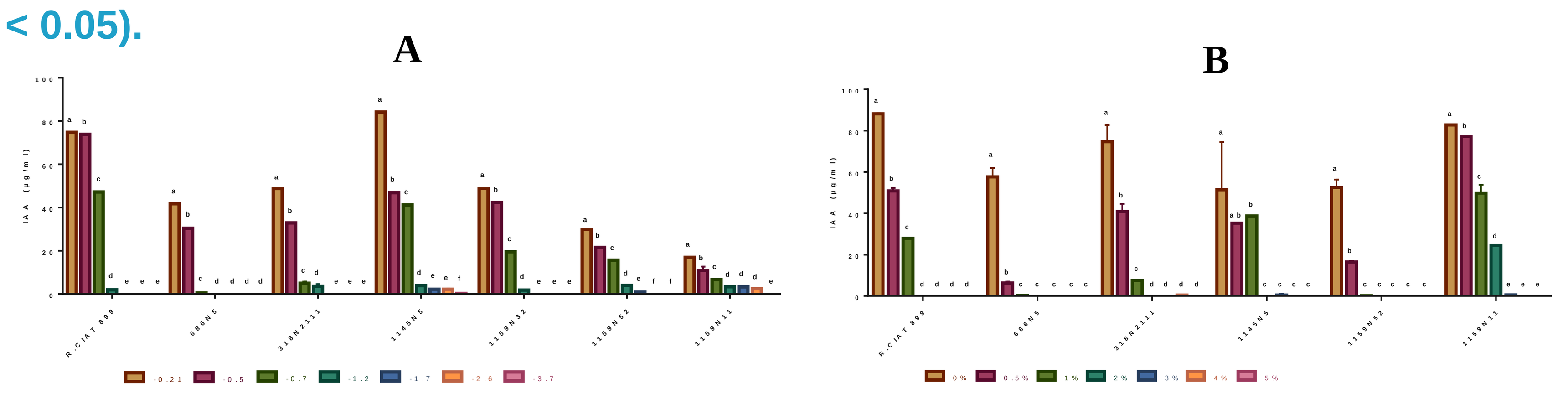

Figure 5 – IAA (Indole Acetic Acid) production (ug/ml) under Drought stress (A) and Salt stress(B). Different letters represent significant statistical differences by using the least significant difference (LSD) (P < 0.05).

Discussion

The results showed a high performance of our Rhizobium strains in terms of IAA production (**strain686N5** with **57.68±4.25** IAA (ug/ml) and Phosphate solubilization activity (PSA ranging between **144.6** and **205.6** P_2O_5 (ug/ml). Saghafi, D. and al., (2019) reported two IAA producer **Rhizobia** (**R281** and **R307**) with an average of IAA production not exceeding **10.2 IAA** (ug/ml). Alikhani and al., (2007) reported that the Iranian Phosphate solubilizing Rhizobia released a rate of P_2O_5 ranging between 88.66-197.1 P2O5 (ug/ml). Moreover, the study showed that salt stress tolerance doesn't necessarily coincide with drought stress tolerance of the same strains. This is the case of the **strain686N5** which showed an extremely high salt-tolerance by growing under 5% against a moderate drought stress tolerance under **ψ=-0.75**MPa. This was previously reported by Mohammad et al., (1991), where they found *Rhizobium meliloti* able to grow better at 616mM NaCl than under **ψ=-1.0** MPa, explaining that salt stress and drought stress tolerance could be due to different mechanisms pathway of tolerance. Interestingly, the assessment of the PGP activities of Rhizobium showed a difference between bacterial viability and the bacterial PGP activity in terms of abiotic stress tolerance. In fact, bacterial PGP activity is interrupted before reaching the bacterial tolerance threshold. This result should integrate a new concept of PGPR screening based on PGP activity abiotic stress tolerance.

Conclusion

The challenge to use the Rhizobacteria as bio-fertilizer for different crops, reside on keeping them in Plant Growth Promotion active status even in harsh conditions. This study showed perfectly that the screening of inoculants based only on their Plant Growth Promotion and stress tolerance performance is not enough. Thus, we propose a new concept of screening based on PGP activity stress tolerance.

Acknowledgment

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References

Alikhani, H. A., Saleh-Rastin, N., & Antoun, H. (2007). Phosphate solubilization activity of rhizobia native to Iranian soils. In First international Meeting on microbial phosphate solubilization (pp. 35-41). Springer, Dordrecht.

Kothamasi D, Kothamasi S, Bhattacharyya A, Kuhad R, Babu, C (2006) Arbuscular mycorrhizae and phosphate solubilising bacteria of the rhizosphere of the mangrove ecosystem of Great Nicobar island, India. Biology and fertility of soils, 42(4), 358-361.

Mohammad, R. M., Akhavan-Kharazian, M., Campbell, W. F., & Rumbaugh, M. D. (1991). Identification of salt-and drought-tolerant Rhizobium meliloti L. strains. Plant and soil, 134(2), 271-276.

Paul M, Rao W, (1971). Phosphate-dissolving bacteria in the rhizosphere of some cultivated legumes. Plant Soil 35, 127–132.

Saghafi, D., Ghorbanpour, M., Ajirloo, H. S., & Lajayer, B. A (2019). Enhancement of growth and salt tolerance in Brassica napus L. seedlings by halotolerant Rhizobium strains containing ACC-deaminase activity. Plant Physiology Reports, 1-11.