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Effect of Inoculation with Consortia of Plant-Growth Promoting Bacteria on Biomass Production of the Halophyte Salicornia ramosissima

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Abstract : Salicornia ramosissima, a halophyte that grows naturally in coastal areas of the northern hemisphere, is often considered the most promising halophyte candidate for extensive crop cultivation and saline agriculture practices. The expanding interest in this plant surpasses its use as gourmet food and includes their potential application as a source of bioactive compounds for the pharmaceutical industry. Despite growing well in saline soils, sustainable and ecologically friendly techniques to enhance crop production and the nutritional value of this plant are still needed. The root microbiome of S. ramosissima proved to be a source of taxonomically diverse plant growth-promoting bacteria (PGPB). Halotolerant strains of Bacillus, Salinicola, Pseudomonas, and Brevibacterium, among other genera, exhibit a broad spectrum of plant-growth promotion traits [e.g., 3-indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophores, phosphate solubilization, Nitrogen fixation] and express a wide range of extracellular enzyme activities. In this work, three plant growth-promoting bacteria strains (Brevibacterium casei EB3, Pseudomonas oryzihabitans RL18, and Bacillus aryabhattai SP20) isolated from the rhizosphere and the endosphere of S. ramosissima roots from different saltmarshes along the Portuguese coast were inoculated in S. ramosissima seeds. Plants germinated from inoculated seeds were grown for three months in pots filled with a mixture of perlite and estuarine sediment (1:1) in greenhouse conditions and later transferred to a growth chamber, where they were maintained two months with controlled photoperiod, temperature, and humidity. Pots were placed on trays containing the irrigation solution (Hoagland's solution 20% added with 10‰ marine salt). Before reaching the flowering stage, plants were collected, and the fresh and dry weight of aerial parts was determined. Non-inoculated seeds were used as a negative control. Selected dried stems from the most promising treatments were later analyzed by GC-TOF-MS for primary metabolite composition. The efficiency of inoculation and persistence of the inoculum was assessed by Next Generation Sequencing. Inoculations with single strain EB3 and co-inoculations with EB3+RL18 and EB3+RL18+SP20 (All treatment) resulted in significantly higher biomass production (fresh and dry weight) compared to non-inoculated plants. Considering fresh weight alone, inoculation with isolates SP20 and RL18 also caused a significant positive effect. Combined inoculation with the consortia SP20+EB3 or SP20+RL18 did not significantly improve biomass production. The analysis of the profile of primary metabolites will provide clues on the mechanisms by which the growth-enhancement effect of the inoculants operates in the plants. These results sustain promising prospects for the use of rhizospheric and endophytic PGPB as biofertilizers, reducing environmental impacts and operational costs of agrochemicals and contributing to the sustainability and cost-effectiveness of saline agriculture. Acknowledgments: This work was supported by project Rhizomis PTDC/BIA-MIC/29736/2017 financed by Fundação para a Ciência e Tecnologia (FCT) through the Regional Operational Program of the Center (02/SAICT/2017) with FEDER funds (European Regional Development Fund, FNR, and OE) and by FCT through CESAM (UIDP/50017/2020 + UIDB/50017/2020), LAQV-REQUIMTE (UIDB/50006/2020). We also acknowledge FCT/FSE for the financial support to Maria João Ferreira through a PhD grant (PD/BD/150363/2019). We are grateful to Horta dos Peixinhos for their help and support during sampling and seed collection. We also thank Glória Pinto for her collaboration providing us the use of the growth chambers during the final months of the experiment and Enrique Mateos-Naranjo and Jennifer Mesa-Marín of the Departamento de Biología Vegetal y Ecología, the University of Sevilla for their advice regarding the growth of salicornia plants in greenhouse conditions.

Keywords : halophytes, PGPB, rhizosphere engineering, biofertilizers, primary metabolite profiling, plant inoculation, Salicornia ramosissima
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