INOCULATION OF MARINE PLANTS WITH MARINE PLANT GROWTH-PROMOTING BACTERIA AND HALOTOLERANT *AZOSPIRILLUM* - A NOVEL APPROACH OF EMPLOYING PGPBs FOR ENVIRONMENTAL USE AND SEAWATER AGRICULTURE.

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Mangroves are tropical and subtropical trees that are the major component of coastal marine-lagoon ecosystems and serve as feeding, spawning, and reproductive areas for numerous economically and ecologically important marine species (9). Tropical mangroves generally self-revegetate after clearcutting. However, in semiarid tropics, after clearcutting, mangroves hardly ever revegetate, and are considered nutrient-deficient ecosystems because they contain low levels of N and P. This apparent nutrient deficiency notwithstanding, these ecosystems flourish with no obvious signs of nutrient deficiency. Nitrogen fixation in mangrove sediment, in the rhizosphere, and associated with aerial roots (8,10) may provide the nitrogen necessary for plant growth, and phosphate-solubilizing microorganisms may supply plants with a sufficient amount of phosphorus (14). Other bacteria that might enhance plant growth by other mechanisms such as those organisms that have been shown to promote the growth of terrestrial crop plants (1,6,7) are unknown in this ecosystem.

It was previously observed that mangrove seedlings usually grow better after inoculation with the diazotrophic filamentous cyanobacteria *Microcoleus chthonoplastes* (3,13). Based on this observation, it was reasoned that mangrove seedlings might also benefit by being inoculated with plant growth-promoting bacteria (PGPB) (2).

Inoculation of axenic black mangrove seedlings in seawater for eight days with either the terrestrial halotolerant plant growth-promoting bacterium *Azospirillum halopraeferens* or *A. brasilense* produced heavy colonization of the root surface. The colonization pattern was different for the two strains. *A. halopraeferens* yielded mainly single cells embedded in a thick sheath, whereas *A. brasilense* produced primarily microaggregates. *A. brasilense* cells were anchored to the root surfaces and to themselves by a network of fibrillar material. Both bacterial strains survived in seawater (approx. 10^4 cfu/mL) for more than 30 days, for 70 days in saline water (*A. brasilense*), and colonized mangrove roots at a high population density. *A. halopraeferens* was a better root surface colonizer, whereas the *A. brasilense* population was greater throughout the entire root (11). A mixed culture of the N_2-fixing *Phyllobacterium* sp. and the phosphate-solubilizing bacterium *Bacillus licheniformis* improved nitrogen (^{15}N) incorporation in leaves and improved seedling development of black mangroves (12). These works are the initial stages of study designed to assess the feasibility of using plant growth-promoting bacteria for the inoculation of marine plants for environmental purposes.

Seawater agriculture is defined as growing salt-tolerant crops on land using water pumped from the sea for irrigation (4). Because none of the top five plant species consumed by man can tolerate high levels of salt, seawater agriculture would only be feasible if salt-tolerant plants that yield useful products at levels high enough to justify the expense of pumping and transporting water from the sea could be cultivated. Our working hypothesis was based on the assumption that instead of breeding a common high yield crop for salt tolerance, it may be easier to select and domesticate wild plants that already have high salt tolerance and possess desirable crop characteristics. Inoculation with PGPBs may help to improve crop yields.

Species of the shrub-like halophyte seaweed *Salicornia* are grown widely in climates ranging from temperate to tropical. *Salicornia bigelovii* is an annual, leafless, fast growing, succulent halophyte found along the seacoast of Sonora and Baja California, Mexico. Because *S. bigelovii* produces oilseeds and a high yield of foliage, it is being evaluated as a new forage and oilseed crop for salt-water and seawater-irrigated agriculture in the coastal deserts of Mexico and the USA (5).

Bacteria with confirmed plant growth-promoting capabilities have not yet been isolated from *Salicornia* plants. Some species of *Azospirillum* can survive well in seawater when inoculated on mangrove seedling roots (11). In addition, several potential marine PGPBs (N_2-fixing and phosphate-solubilizing bacteria) were isolated from mangrove seedlings (14).

Inoculation of *S. bigelovii* with eight species of halotolerant PGPBs and grown in seawater-irrigated pots under environmental conditions native to the plant’s habitat, resulted in significant plant growth promotion by the end of the growing season, 8-11 months later. Statistical analyses demonstrated that inoculation with terrestrial halotolerant *Azospirillum halopraeferens*, a mixture of two halotolerant *A. brasilense* strains, a mixture of marine *Vibrio aestuarianus* and *V. proteolyticus*, or a mixture of marine *Bacillus licheniformis* and *Phyllobacterium* sp. significantly increased plant height and dry weight at the end of the season. Some of the bacterial strains also increased the number of side branches and the length of the spikes. The bacteria did not affect the number of seeds or their weight. Inoculation with the mangrove associated cyanobacterium *Microcoleus chthonoplastes* previously shown to have positive effects on development of mangrove plants (3,13) had no effect on *Salicornia* plant foliage variables (Fig. 1). At the end of the growing season, the nitrogen and protein content of the plant foliage were significantly reduced by bacterial inoculation, however, the nitrogen and protein content of seeds significantly increased. The phosphorus content in foliage increased significantly in plants treated with all these bacteria with the exception of *M. chthonoplastes,* whereas the total lipid content of foliage increased significantly only when plants were inoculated with either a mixture of *A. brasilense* strains or with *M. chthonoplastes* (Fig 2). In three inoculation treatments, palmitic acid in seeds significantly increased and linoleic acid significantly decreased, improving oil quality.

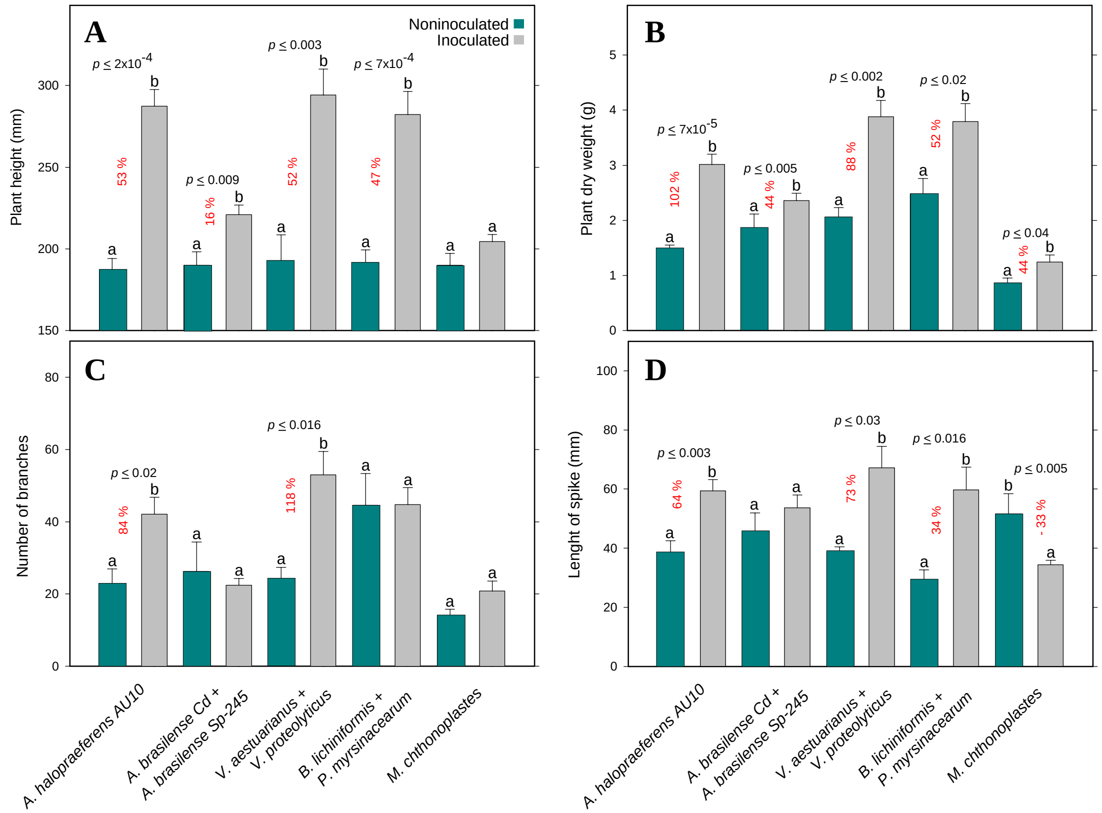

Fig. 1. Effect of inoculation with several plant growth-promoting bacteria on various morphological characteristics of *Salicornia bigelovii* plants at the end of the growing season. A) plant height, B) dry weight, C) number of branches, d) length of spike. Pairs of values (inoculated and uninoculated) in each subfigure that differed significantly at a *P* level denoted above the pair are indicated by different lower case letters. Vertical numbers above each pair indicate significant increase (or decrease), in percentage, of inoculated plants as compared to uninoculated plants. Bars represent SE.

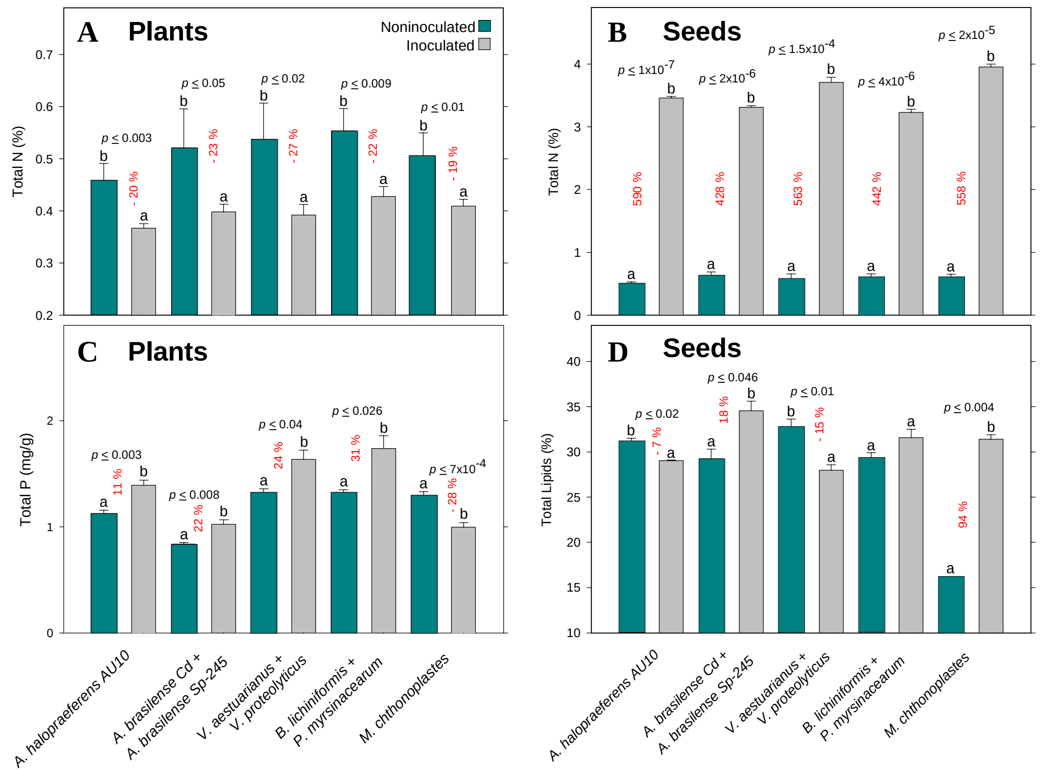

Fig. 2. Effect of inoculation with several plant growth-promoting bacteria on the chemical composition of *Salicornia bigelovii* foliage and seeds at the end of the growing season. A) Total N content of foliage, B) total N content of seeds, C) total P content of foliage, D) Total lipid content of seeds. Pairs of values (inoculated and uninoculated) in each subfigure that differed significantly at a *P* level denoted above the pair are indicated by different lower case letters. Vertical numbers above each pair indicate significant increase (or decrease), in percentage, of inoculated plants as compared to uninoculated plants. Bars represent SE. Absence of a bar in any column indicates negligible SE.

This study demonstrates the feasibility of using bacteria to promote the growth of halotolerant plants cultivated for forage and seed production in seawater-irrigated agriculture.

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