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"Microorganisms for future agriculture"

Endophytic diazotrophic bacteria obtained in flooded rice cultivars: potential for utilization in biological nitrogen fixation.

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ABSTRACT

Forty-five strains of endophytic diazotrophic bacteria (EDB) with capacity for biological nitrogen fixation (BNF) were isolated from leaves, stems and roots of flooded rice cultivars BRS-7 Taim and BRS Pelota,. Among the selected EDB's, fifteen showed a positive interaction with BRS Fronteira, BRS Querência and BRS Pampa to fix nitrogen, evidenced mainly by higher multivariate vegetative development. The polyphase characterization of strains will be necessary to identify the bacteria.

INTRODUCTION

In the Rio Grande do Sul state, located at the southern part of Brazil, approximately 1.07 million of hectares are cultivated with flooded rice by year. This region contributes with 67 % of the cereal produced in the country. Nitrogen (N) is the nutrient that provides the highest productivity responses to irrigated rice. Intensive use of nitrogen fertilizers, coupled with the rising cost of chemical fertilizers, has generated environmental concerns and questions about sustainability amongst rice producers and the society. Thus, the biological nitrogen fixation (BNF) assumes greater importance and could be a innovation to optimize N use in the field rice. Forty-five strains of endophytic diazotrophic bacteria (EDB) were isolated from leaves, stems and roots of BRS-7 Taim and BRS Pelota flooded rice cultivars showed capacity for BNF (Mattos *et al.*, 2010). In this paper, we report the selection of EDB's and their interaction with three rice cultivars.

MATERIAL AND METHODS

Greenhouse experiments performed as described in Mattos *et al.* (2011). Completely randomized factorial design, involving three cultivars combined with 20 treatments, and positive (with nitrogen) and negative (free nitrogen) controls. Multivariate analysis of variance (MANOVA) and Tukey test at 5% were performed for multiple comparisons of means. The first canonical discriminant function of Fisher, called multivariate vegetative development (MVD), was generated. The parameters evaluated were: length of shoots and roots, stem diameter, chlorophyll content, fresh weight of leaves and roots, dry weight of shoots and roots.

Strains arising from the Collection of Multifunctional Temperate Climate Microorganism Cultures 45 EDB's, grown in NFb with film formation were inoculated (cell concentration of 10^5 - 10^8 CFU mL⁻¹) in flooded rice cultivars BRS Fronteira, BRS Querência and BRS Pampa. Strains characterization: cell morphology, physiological and biochemical characteristics.

RESULTS AND DISCUSSION

Bacteria CMM174, CMM175, CMM176, CMM177, CMM178, CMM179 with potential for BNF in rice cultivar BRS Pampa highlighting CMM179 endophytic diazotrophic stem associative genotype.

EDB's differed in their ability to promote the growth of BRS Pampa. There was a significant contribution by six strains isolated from stalk of BRS Pelota (Mattos *et al.*, 2010). CMM 178 and CMM176 belong to the genus *Bacillus*, while the other strains are

Gram-negative and not forming endospores. Positive effects of bacteria CMM171, CMM180, CMM192, CMM193 CMM194 were observed by multivariate vegetative development (MVD), where the variables related to the roots indicated a stimulation of root growth, induced probably by the production of hormones. CMM179 endophytic diazotrophic stem emerged from associative farming, increasing 354% in the MVD when compared to the treatment without nitrogen. Bacterial consortium formed with CMM174, CMM175 and CMM179 were associated with the rice lines AB10005, AB09021, AB09006, AB09002 and AB09044, evidenced by increased of dry matter of the shoot, and by positive changes in the Relative Index of Chlorophyll leaves (Fagundes *et al.*, 2011).

Diazotrophics CMM 194, CMM 195, CMM 196, CMM 197, CMM 182, CMM 183, CMM 200, CMM 203 and CMM 205 with positive interaction between rice cultivars BRS Fronteira and Querência and bacteria inoculated to FBN.EDB's, isolated from the stems, leaves and roots of BRS-7 Taim and BRS Pelota differed in the interaction with cultivars. The establishment of a good inoculum endophyte plants in rice is dependent on the ratio bacteria / genotype (Baldani *et al.*, 2000). The Fischer canonical discriminant function, based on the synthesis of the information contained in the length of shoots and roots and stem diameter variables, forms three groups for each cultivar. CMM 194, CMM 195, CMM 196, CMM 197, CMM 182, CMM 183, CMM 200, CMM 203 and CMM 205 components of group 1 had a higher ability to colonize plants, demonstrating a positive interaction between cultivars and inoculated bacteria to FBN (Figure 1). The nine strains belong to the group of Gram-negative bacteria, exhibiting morphological, physiological and biochemical differences.

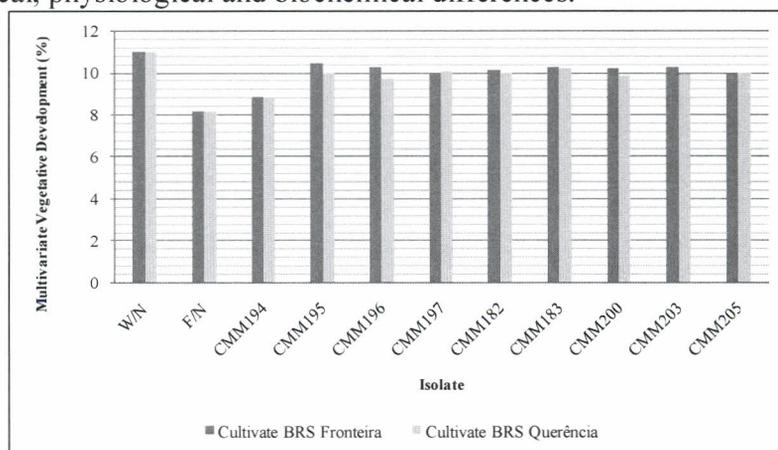


Figure 1. Comparison of the interaction between nine strains and two cultivars. Multivariate Vegetative Development data in percentages; length of shoots and roots and stem diameter evaluated simultaneously on the same plant.

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