Effect of inoculation of the rhizobacteria *Azospirillum brasilense* FP2 *nifH::gusA* and HM053 *nifH::gusA* strains in barley


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INTRODUCTION: Nitrogen-fixing bacteria of the genus *Azospirillum* colonize the rhizosphere of economically important crops exerting beneficial effects on plant growth. The knowledge and understanding of plant–microbe interactions are limited, mainly due to difficulties in studying bacterial populations at root surfaces or enclosed in plant tissues. Reporter genes like *gusA* are powerful molecular tools used in ecological studies. MATERIAL AND METHODS: *A. brasilense* FP2 and HM053 single recombinants (SR, *nifH*+ and *gusA*+) and double recombinants (DR, *nifH* and *gusA*+) mutant strains containing a chromosomal *nifH-gusA* fusion were evaluated regarding their nitrogenase activity. In addition, to determine the colonization capacity of these mutants, barley (*Hordeum vulgare* L. CAUÊ) was inoculated with the strains and plant development was followed during 35 days under controlled conditions. RESULTS AND DISCUSSION: Analyses of colonized plants showed that both strains FP2 and HM053 carrying the *nifH::gusA* fusion expressed the *nifH* gene on the surface of the roots. Moreover the DR mutants were not able to fix nitrogen, confirming the inactivation of *nifH*. The FP2-SR mutant fixes nitrogen only in the absence of ammonium, while HM053-SR fixed nitrogen even in the presence 10 mM ammonium chloride. No statistical difference was detected among barley plants 14 or 21 days after inoculation with the *A. brasilense* strains. However, 35 days after inoculation, plants treated with strains FP2, HM053 or their SR derivatives strains presented longer roots (Tukey 5% test) than the non-inoculated controls or inoculated with the DR mutant strains. CONCLUSION: Our results suggested that nitrogen fixation is an important plant-growth promoting factor, since longer roots were obtained when barley were inoculated with the nitrogenase active (*nifH*+) strains but not with the *nifH* DR mutant strains. The *gusA* expression allowed checking the presence of the inoculated *A. brasilense* mutant strains inside and outside the barley roots during the assay.

Key words: reporter gene, nitrogen fixation, inoculant, plant growth

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