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Induced colonization of GFP- labeled *Azorhizobium caulinodans* ORS 571 in rice roots

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Rice, being the most highly consumed cereal crop in Sri Lanka demand a very high input of nitrogen fertilizer for cultivation. Use of nitrogen fertilizer over a long period is known to cause many environmental and socio-economic problems. In the search of an alternative for nitrogen fertilizer, Biological Nitrogen Fixation (BNF) is an excellent alternative. Successful colonization of rice roots by a nitrogen-fixing bacterium is a prerequisite for producing a nitrogen fixing rice plant. This study aims at inducing colonization of *Azorhizobium caulinodans* ORS 571 in the roots of rice variety BG 359 (i.e. cultivar popular in Sri Lanka) and determining the optimum conditions for maximum efficiency of colonization, in terms of inoculum volume, frequency of inoculation, best time interval for the detection of the bacterium inside the rice plant in the presence of a signalling molecule Naringenin. The bacterium *A. caulinodans*, being a free-living nitrogen fixer, and having the ability to tolerate oxygen 3% v/v is advantageous over other nitrogen fixers in non-symbiotic nitrogen fixation. The bacterium was labelled with a green fluorescent protein marker (GFP) for reliable and accurate detection *in vivo*. GFP- labelling was carried out by inserting the *gfp* gene containing plasmid pBBR5-hem-gfp5-S65T into *Azorhizobium caulinodans* ORS 571, with the help of a helper plasmid (pRK2013) by tri parental mating. The degree of colonization was measured through fluorescence of GFP by computer software (ZEN 2012). The colonization increased up to the 25^{th} day and then decreased giving very low intensity measurements. It was revealed that 5ml (10^8 cells/ ml) of the culture added twice a week for 15 days results in best conditions for colonization by statistical analysis. Optimum conditions can be used for future experiments of nitrogen fixation in rice.

Key words: *Azorhizobium caulinodans* ORS 571, BNF, detection *in vivo*, GFP

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