# A nitrogen fixing association for rice with the diazotroph *Azorhizobium caulinodans* to reduce urea fertilizer in rice cultivation

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# Abstract

Nitrogen is the *sine qua non*, or the absolute necessity for cultivation of rice, the staple food of more than half of the worlds population. Inorganic nitrogen fertilizers can have severe negative impacts on the socio economic status and the environment of a country. Finding an alternative to inorganic nitrogen fertilizers is a timely need and is evident through global attention that the issue has received during the past decade. Considering all of the above facts, the main objective of this research was to develop a nitrogen fixing association between rice and the diazotroph *Azorhizobium caulinodans* ORS $571^T$, to reduce the usage of nitrogen fertilizers without affecting the growth or yield of rice. Bacterium *A. caulinodans* possesses certain uniquely beneficial properties (the ability to tolerate 3% v/v oxygen, ability to fix nitrogen in free living conditions etc.), which can benefit non legume plants such as rice through atmospheric nitrogen fixation.

As the first step, the bacterium *A. caulinodans* was labelled with a green fluorescent protein for *in-vivo* detection and the bacterial colonization was determined through green fluorescence intensities. Several different strategies were used to incorporate the bacterium in to the rice plant roots intending to find out the best method of incorporation. It was observed that when *A. caulinodans* was formed in to a biofilm with the rice rhizosphere fungus *Aspergillus* spp. (AAB) and when inoculated in the presence of flavonoid naringenin (Nar) to rice, the rice root colonization (through epifluorescent microscopic data), endophytic colonization and acetylene reduction assay values were significantly ($p < 0.05$) high in comparison to the other strategies used (*A. caulinodans* + Nar, AAB, *A. caulinodans* in water limiting conditions, and *A. caulinodans* only) and hence this strategy was used in all following experiments.

A pot experiment of rice with the treatments 100% nitrogen fertilizer recommendation with no AAB/Nar (Control), 75% of nitrogen fertilizer recommendation + AAB/Nar (75% $N^{AAB/Nar}$), 50% $N^{AAB/Nar}$, 25%$N^{AAB/Nar}$ and 0%$N^{AAB/Nar}$ was conducted for evaluating rice root colonization, nitrogen fixation (NFIX) and nitrogen yield (NYIELD). The 60 day old plants showed the highest root colonization, NYIELD and NFIX in the treatment 50%$N^{AAB/Nar}$. Plants treated with 50%$N^{AAB/Nar}$ and harvested at 105 days had the highest grain yield, NYIELD and NFIX. Thereafter, two field experiments were conducted with the treatment 50%$N^{AAB/Nar}$ to find out whether the developed biofertilizer acts in field conditions in the same manner as the pot experiment. The growth and yield data of the field experiments also resulted in significantly ($p < 0.05$) higher or similar results for 50%$N^{AAB/Nar}$ compared to that of the control (100% N).

According to the positive results obtained in the pot and field experiments it was concluded that, 50% of nitrogen fertilizer recommended coupled with *A. caulinodans–Aspergillus* spp. biofilm in the presence of naringenin is a highly effective biofertilizer which can reduce adverse effects of inorganic nitrogen fertilizers and is eco - friendly, applicable and cost effective.