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An *Azotobacter* with partially deleted *nifL* and constitutive *nifA* can save a good amount of urea fertilizer without affecting wheat yield

Hirendra K Das[1], Umesh K Bageshwar[1], Madhulika Srivastava[1], Peddisetty Pardha-Saradhi[2], Sangeeta Paul[3], Sellamuthu Gothandapani[4] and Ranjeet S Jaat[4]
[1]Jawaharlal Nehru University, India
[2]Delhi University, India
[3]Indian Agricultural Research Institute, India
[4]National Research Centre for Plant Biotechnology, India

Soil bacteria belonging to the genus *Azotobacter* can reduce atmospheric nitrogen into ammonia, which can be assimilated by plants. About 20 genes are involved in the process of nitrogen reduction. Expression of all these genes is dependent on a positive regulator *NifA*. In the presence of ammonia, the negative regulator *NifL*, gets activated and interacts with *NifA* to neutralize it. As a result no nitrogen is reduced by wild type *Azotobacter* when ammonia is formed or produced by nitrogenous fertilizers. In two species of *Azotobacter* studied by us, the *nifL* and *nifA* genes were present in the same operon, *nifL* being proximal and *nifA* distal to the promoter. The *nifL*A promoter was also regulated by ammonia. We partially deleted the *nifL* gene and inserted a constitutive promoter there, thus bringing the *nifA* gene under its control. Ammonium only marginally affected acetylene reduction (a measure of nitrogen reduction) by the engineered *Azotobacter* strain. When wheat seeds were inoculated with the engineered strain and sown, the crop yield was enhanced by 60%, without any application of urea or other chemical nitrogenous fertilizer. The crop yield, however, was enhanced only by 10% when the wheat seeds were inoculated with the wild type strain. When we applied urea, the wheat plants arising from the seeds inoculated by the engineered strain, could yield the same amount of crop using ~85 kg less urea (~40 kg less nitrogen) than the usual ~257 kg urea (~120 kg nitrogen) per hectare. Wheat plants from the seeds inoculated with the engineered strain had much higher dry weight and nitrogen content and assimilated molecular 15 N much better than plants from seeds inoculated with the wild type strain. Inoculation of the wheat seeds with the engineered strain did not adversely affect the microbial population in the rhizosphere soil.

hirendas@hotmail.com