

Nitrogen Symbiosis in Cereals

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Increasing global population, which is 7.5 billion today, is predicted to rise to over 9 billion by 2050 (UN DESA, 2013) and changes in diet are driving up the need for food. Cereals are the important staple food throughout the world, especially rice, wheat and maize. Intensive agricultural systems that derive much of global food production are far from sustainable and a 'business as usual'. Approaches to meet the increased demand for food will lead to significant depletions in natural resources. The production and application of chemical fertilizer is the major source of pollution as well as the major use of energy in agricultural systems.

Nitrogen is an essential plant nutrient. It is the nutrient that is most commonly deficient, contributing to reduced agricultural yields throughout the world. Broadly nitrogen supply can be done through external inputs as organic or inorganic nitrogen, mainly as fertilizers or through naturally occurring biological nitrogen fixation. Typical nitrogen-use efficiencies for wheat, rice, and maize indicate that around 66% of this nitrogen is lost to the environment (Raun and Johnson, 1999), either in the form of nitrous oxides, which are potent greenhouse gases, or as soluble nitrates that find their way into aquatic systems (Glendinning et al., 2009). Considering losses it is better to reduce dependency on nitrogen fertilizers. Molecular nitrogen or dinitrogen (N₂) makes up four-fifths of the atmosphere, but is metabolically unavailable directly to higher plants or animals. It is available to some microorganisms through biological nitrogen fixation (BNF) in which atmospheric nitrogen is converted to ammonia by the enzyme nitrogenase.

Loss of nitrogen can be overcome by various processes like fertilizer nitrogen management, organic nitrogen management, nitrogen use efficient genotypes and biological nitrogen fixation. But loss in case of BNF is less compared to other management processes. Since, in agricultural ecosystems, the BNF usually does not exceed the nitrogen requirement of

the ecosystem, the fixed nitrogen gets used up by the system and does not add to the pollution.

Extending the process of BNF to cereals is of immense research interest because nitrogen derived from fixation is considerably more economical and ecofriendly as the fixed nitrogen is not as susceptible to losses as that of fertilizer N. Major limitation of associative nitrogen fixation in cereals is that bacteria in the rhizosphere of plants utilise the products of nitrogen fixation for their own growth, but release little while they are alive (Berkum and Bohlool, 1980) which is in contrast to symbiotic associations like legume- rhizobium symbiosis.

The possibilities of extending the host range of Rhizobia to non-legumes were encouraged by the discoveries that *Rhizobium* forms nodules in *Parasponia* spp.

Approaches for achieving nitrogen fixation in cereals

Non nodular approaches

This improves the associations between cereals and nitrogen-fixing soil bacteria which includes achieving colonization and invasion of cereals roots by suitable diazotrophs.

- Endophytic association- screening of germplasms for colonization by diazotrophs.

The culturable bacterial diazotrophs were isolated from endophytic tissue of maize: seed, root, stem, and leaf. All isolates were able to grow on N-free semisolid medium. Eleven bacteria isolates showed nitrogen- fixing capacity which was measured by acetylene reduction assay (Montanez *et al*, 2009).

- Investigation of root morphogenesis in relation to invasion

As we know that root nodule formation is closely related with lateral root formation, investigations were carried out by many scientists to

observe morphogenesis and lateral root formation in many cereals upon inoculation with strains of rhizobia.

c) Use of rhizobial inoculants which nodulate crops

The study of the interaction of *Azorhizobium caulinodans* (ORS571) with both rice and wheat has shown significant levels of nitrogen fixation, as assessed using acetylene reduction assay, and there was a correlation of this nitrogen fixation with the invasion of emerging lateral roots by 'crack entry' and the initiation of lateral root nodule formation (Cocking *et al.*, 2001).

Nodular Approaches

a) Interactive responses between flavonoids and rhizobia.

The flavanone, naringenin, when applied at both 10^{-4} and 10^{-5} M concentrations, significantly stimulated the colonization of LRCs (lateral root cracks) of wheat by *Azorhizobium caulinodans* ORS571, but both daidzein and luteolin had no effect (Webster *et al.*, 1997).

nif gene transfer

To ensure expression of nitrogenase, protection of nitrogenase from inactivation by oxygen, and an energy supply for N_2 fixation without compromising yield, transformation of *nif* genes in cereals is one of the options.

The engineering of plants capable of fixing their own nitrogen requires the coordinated expression of 16 *nif* genes. Plastids may provide a favourable environment for *nif* gene expression. The introduction and expression of one of nitrogenase components, the Fe protein, in transgenic tobacco was

achieved although results were not encouraging, they do provide important clues for future approaches (Dixon *et al.*, 1997).

Significant progress has been made towards the induction of rooting modifications in cereals that resemble legume root nodule organogenesis. These findings will enable the cereals to fulfil part of their N requirements leading to savings in fertilizer inputs and consequently the environment.

References

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