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DEVELOPMENTS IN NONLEGUME N_2-FIXING SYSTEMS

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A large number of cereals and grasses have been shown to support nitrogen fixation, measurable by acetylene (C_2H_2) reduction, N balance, ^{15}N isotope dilution and more directly, $^{15}N_2$ incorporation studies. The spiralling increase in the prices of nitrogenous fertilizers during the energy crisis, attracted the worldwide attention of policy makers and researchers to this area of research. However, this enthusiasm has declined in last five years. In this paper, important developments in the field of nitrogen fixation associated with non legumes are reviewed, and the work at ICRISAT on cereal nitrogen fixation is briefly discussed.

The distribution of nitrogen-fixing bacteria associated with nonlegumes is widespread in nature. The work on microbiology of the association has been reviewed (1, 2). The issue of choosing the right cultural conditions and the culture media is complicated because several types of bacteria, that are able to fix nitrogen, proliferate in the rhizosphere of different plants. The population and number of colony types varied with the carbon source in a culture medium and increased on addition of yeast extract (50-100 mg L^{-1}) in a medium. A clear rhizospheric effect for the number of nitrogen fixers has been observed. In our studies, higher most probable number (MPN) counts and a number of active isolates of nitrogen fixers associated with pearl millet, were observed in a combined carbon source medium (containing sucrose-5 g, mannitol-5 g, malic acid-5 g, K_2HPO_4-0.8 g, KH_2PO_4-0.2 g, NaCl-0.1 g, Na_2MoO_4. $2H_2O$-0.0025 g, $MnSO_4$ $2H_2O$-0.01 g, yeast extract-0.1 g, glutamic acid 0.37 g, Fe EDTA-4 ml of 1.64% aquaeous solution, 3 ml of bromothymol blue 0.5% in ethanol, distilled water 1000 ml and pH 6.8) than in malate medium. However, counts of heterotrophic bacteria, using planting method were higher in a N-free malate medium. Significantly, higher MPN counts and a number of heterotrophs able to grow on malate and combined carbon source media were recorded from washings of pearl millet roots than from either the rhizosphere soil or washed root macerate samples. Similarly, a number of heterotrophs and MPN counts of associative nitrogen fixers were found to be higher at the flowering stage of the pearl millet plant and to decline on maturity. The counts recorded at the maturity stage were higher than those observed at early stages of plant growth. A survey of 200 sites in the traditional millet growing areas in northwestern India indicated MPN counts varying from 100-100,000 g^{-1} soil, in N-free malate and sucrose semi-solid media.

Among several types of nitrogen-fixers observed, the members of the genus **Azospirillum** (**A. brasilense**, **A. lipoferum** and **A. amazonense**) were recently studied. Azospirilla are generally found wherever they are sought and can use a wide variety of carbon and energy sources for their growth on combined N or N_2. There are two main physiological types within the genus of **Azospirillum**. One group has an oxidative metabolism and the other has the ability to ferment certain sugars, producing acid. These bacteria are very versatile in their nitrogen transformations. In addition to their nitrogen-fixing ability certain strains denitrify under anerobic conditions and could also assimilate NH_4, NO_3 or NO_2 (2, 3). Another trait observed in **Azospirillum** is the presence of H_2 uptake (hydrogenase) activity to recycle the H_2 produced by nitrogenase (4). In **A. brasilense** (SP7) grown anerobically on N_2O or NO_3, O_2 dependent H_2 uptake was inhibited irreversibly by NO_2, NO, and C_2H_2 and reversibly by CO (5). Serologically, **A. brasilense** and **A. lipoferum** are different and **A. lipoferum** represented a more homogenous group in respect to fluorescent antibody (FA) reactions. In contrast, **A. brasilense** consisted of at least 3 subgroups (6, 7). Using the immuno diffusion test, both the species produced at least one heat labile precipitation band. At ICRISAT, using enzyme-linked immunosorbent assay (ELISA), we have observed that azospirilla are serologically distinct from other nitrogen-fixing bacteria. **A. brasilense**, **A. lipoferum** and **A. amazonense** are serologically distinct from each other and strains of **A. lipoferum** form a homogenous group serologically, whereas **A. brasilense** strains form a heterogenous group. ELISA can be used for identification of **A. lipoferum**, and also for enumeration of azospirilla in pure cultures and peat inoculants.

The role of various factors, (namely plant genotype, radiation, soil temperature, moisture, and combined N), influencing nitrogen fixation is discussed. Seasonal and diurnal variations in nitrogenase activity have been observed and the activity is higher in wet and warmer soil (32-35 C). The activity is stimulated by low levels of combined N (10 ppm or 20 kg ha^{-1} N) and inhibited by higher N levels (8). Plant genotypes of several crops varied for high and low nitrogenase activity (9) and high nitrogenase activity (upto 5800 nmol C_2H_4 $plant^{-1}$ h^{-1}) over the seasons in lines of pearl millet and sorghum have been observed (8). A large plant to plant variability, ranging from 0-1900 nmol C_2H_4 $plant^{-1}$ h^{-1} in the Ex-Bornu population, has been observed. Recently, it has been reported that selfed or hybridized lines of pearl millet possessing high nitrogenase activity showed higher nitrogenase activity, bacterial counts and higher loss of ^{14}C from the roots than the groups obtained from low acetylene reduction activity (ARA) lines (10). It might be possible to develop genotypes that stimulate more nitrogen fixers in their rhizosphere due to genetically based enchancement of specific biochemical characteristics.

Such a plant breeding approach, if successful, will eliminate the need for artificial inoculation.

Till such genotypes are available, the route of artificial inoculation of plants with nitrogen-fixing bacteria has to be adopted to obtain increased yields, under low fertility situations. Several studies conducted with different crops inoculated with different bacteria showed increased yields that may or may not be statistically significant, and sometime negative responses. A review of the voluminous Russian literature on inoculation with **Azotobacter** concluded that positive significant effects occurred in about a third of the trials; that some horticultural crops responded better than cereals; and that best results were obtained in soils rich in organic matter, with mineral fertilizer N added (11). In the last decade, several inoculation experiments with different crops using **Azospirillum** spp. were conducted and were reviewed (2). Several experiments in Egypt, India, Israel, and USA showed positive benefits of inoculations under field conditions. The inoculation effects were more pronounced with lower levels of N fertilizer added. However, similar benefits in the presence of high levels of N added are also reported from Israel. During 1982-84, 5 out of 9 trials with pearl millet cultivars conducted at ICRISAT Centre and other locations in India, showed significantly ($P = \leq 0.05$) increased grain yields across the cultivars. However, significant interaction between host cultivars and bacterial strains was observed in only one trial. Several studies suggest that it is possible to increase cereal crops yields by inoculation with nitrogen-fixing bacteria in countries where cereals are only partially fertilized, or not fertilized at all. We need more precise knowledge of the agronomic practices that help increase nitrogen fixation under normal situations and with inoculation. Information on the role of organic amendments, synergistic levels of combined N, appropriate form and method of application, effect of other plant nutrient elements on nitrogen fixation will help derive maximum possible benefits from associative nitrogen fixation.

Synergistic host responses in terms of increased yield due to dual inoculation with nitrogen-fixing bacteria and vesicular arbuscular mycorrhizal (VAM) fungi have been reviewed (12). In studies with tomato, mycorrhizal infection increased the **A. chroococcum** population in the rhizosphere that was maintained at a high level for a longer time and **A. chroococcum** enhanced infection and spore production by the mycorrhizal fungus (13). In sorghum, dual inoculation with **Azospirillum** and VAM resulted in increased VAM colonization and biomass, while the N input due to **Azospirillum** decreased, possibly due to competition for carbohydrates (14). Similarly, in barley, dual inoculation with **A. brasilense** and VAM produced increased grain and biomass yield in pot culture studies.

However, inoculation with **Azospirillum** did not increase mycorrhizal root infection (15).

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