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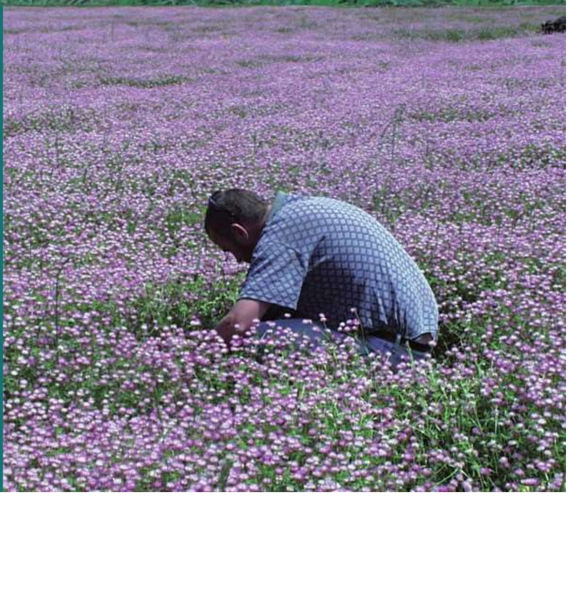
Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture



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INOCULANTS FOR SUGAR CANE: THE SCIENTIFIC BASES FOR THE ADOPTION OF THE TECHNOLOGY FOR BIOFUEL PRODUCTION

V. M. Reis[1], A. L. M. de Oliveira[2], M. F. da Silva[3], F. L. Olivares[4], J. I. Baldani[1], R. M. Boddey[1] and S. Urquiaga[1]

[1]Embrapa Agrobiologia, BR 465, km 47 and km 07, 23890-970 Seropédica, RJ, Brazil; [2]Departamento do Biologia, Universidade Estadual de Londrina, Londrina, PR, Brazil; [3]Universidade Federal Rural do Rio de Janeiro, Instituto de Agronomia, doutorado em Ciência do Solo, km 47, Seropédica, RJ, Brazil; [4]Centro de Biociências e Biotecnologia, UENF, Campo dos Goytacazes, RJ, Brazil
(Email: veronica@cnpab.embrapa.br)

Sugar cane is one of the graminaceous species that can obtain most of its nitrogen from biological N_2 fixation (BNF). Available evidence suggests that BNF contributions are dependent on cane variety and soil properties. Using the ^{15}N natural-abundance technique, the proportion of N derived from BNF may range from zero to 70%. The impact of saving just half of the nitrogen fertilizer used on the crop in Brazil (mean of 60 kg N ha^{-1}) would be approximately 150,000 mg of fertiliser N per year.

Sugarcane is propagated vegetatively via stem pieces (setts) and a considerable quantity of sucrose is present and starts to decompose as the sett germinates. This decomposition pathway liberates sub-products normally used by different microorganisms, including diazotrophs, present in the soil and plant. The selection for use of an inoculant containing a mixture of diazotrophic bacteria, based on the knowledge of the ecological behaviour of the species involved (Oliveira et al., 2006), is the key to obtaining high BNF contributions to this crop.

The experiments performed in Brazil used a mixture of five different diazotrophic species all isolated from sugarcane. These were: *Gluconacetobacter diazotrophicus* strain BR 11281^T; *Herbaspirillum seropedicae* strain BR 11335; *H. rubrisubalbicans* strain BR 11504; *Azospirillum amazonense* strain BR 11145, and *Burkholderia tropica* strain BR 11366. All strains were deposited in the diazotrophic bacterial collection of Embrapa Agrobiologia. This mixture was applied to two sugarcane varieties, SP 70-1143 and SP 81-3250, grown under commercial field conditions at three sites with contrasting soil types; an Alfisol, an Oxisol and an Ultisol, which are equivalent to low,

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medium and high natural fertility, respectively. In the first trial, sterile micro-propagated plantlets were inoculated with the diazotrophs (as described by Reis et al., 1999). In the Alfisol, the response to inoculation of the stem yield, dry matter and N accumulation of the variety SP70-1143 (previously identified as high in BNF) was equivalent to the effect of the usual annual nitrogen fertilization. The plants were grown without N fertilizer for three consecutive years (a plant crop and two ratoons) and the ^{15}N data indicated that inoculation promoted a significant increase in the contribution of BNF.

Because sugarcane is normally propagated by setts, the next step was to perform a new procedure to introduce these five diazotrophs into stem pieces by immersion, after the heat treatment (50°C for 30 min) normally used to control ratoon-stunting disease. The strains were grown individually, then adjusted to the same cell density (10^8 cells mL^{-1}), and mixed before planting the setts. Two new sugarcane varieties, RB 72-454 and RB 86-7515, were used to test this inoculation procedure. The assay was performed using plastic trays containing a mixture of vermiculite + sand (2:1) and, after 50 days, plant colonization and biomass accumulation were evaluated. The bacterial population colonized plant tissue in numbers higher than 10^6 cells g^{-1} fresh weight. Root biomass accumulation was higher in the presence of bacterial application. This inoculation procedure can be used to select bacterial strains in a rapid trial and adapted to new varieties of sugarcane, which are regularly being introduced to the cropping systems.

Using this methodology, Medeiros et al. (2006) evaluated the effect of inoculation of *G. diazotrophicus* and the addition of increasing amounts of two sources of mineral nitrogen (ammonium sulphate and calcium nitrate) on the population of this diazotroph, its nitrogenase activity (acetylene reduction), and accumulation of N by two sugarcane hybrids, SP 701143 and SP 792312. The results showed that both varieties differed in the form of nitrogen they prefer to take up from the soil. In both varieties, the addition of increased doses of ammonium and nitrate decreased the population of *G. diazotrophicus* but, in the variety SP 70-1143, the inhibition was more pronounced in the presence of calcium nitrate. Acetylene-reduction activity was inhibited in both varieties, especially in variety SP 79-2312, in the presence of either of the nitrogen sources. These results demonstrate that the interaction of sugarcane and diazotrophic bacteria is sensitive to nitrogen application in a similar manner to legume symbioses.

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