NITROGEN NUTRITION OF SUGARCANE WITH FERTILIZERS OR DIAZOTROPHIC BACTERIA

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Nitrogen is required in large quantities for biomass production. Around 23% of the fertilizer N in Brazil is used in sugarcane. Besides being the most expensive plant nutrient, N fertilizers are an important component of the environmental budget of biofuel production. It is estimated that synthesis of N fertilizer accounts for about 25% of all the fossil energy spent in field operations for ethanol production from sugarcane in Brazil. Emission of nitrous oxide, a potent green house gas associated with fertilizer use, also adds to the environmental costs of ethanol.

There are evidences that biological nitrogen fixation (BNF) is responsible for supplying part of the N required by sugarcane plants because several diazotrophic microorganisms have been isolated in that crop. Besides, the amounts of N fertilizer applied to sugarcane in many cases do not replenish the N removed from the fields with the harvest or lost as part of management practices. However, old sugarcane fields generally do not show signs of soil degradation.

The actual contribution of BNF to sugarcane under field conditions is controversial. Some authors have expressed their view that BNF is of little relevance for sugarcane N nutrition. However, it is generally recognized that BNF presents great potential especially in Brazil where many studies have shown promising results.

BNF is affected by plant variety, bacteria species, and plant-bacteria interactions. An inoculant produced with five strains of N2-fixing diazotrophs was recently developed by Embrapa but it has not been extensively tested under field conditions. This project has the objective of studying the contribution of BNF to sugarcane production compared with the use of synthetic N fertilizer under different soils, environments, and sugarcane cultivars, evaluating the emission of N_2O from sugarcane fields fertilized with N, and testing an inoculant produced with endophytic bacteria. At the same time new N_2 fixing organisms are being searched for that are adapted to the sugarcane growing conditions of the State of São Paulo. The project will be complemented with studies on genetic traits of sugarcane associated with the capacity of N_2 fixation, which may help to obtain new varieties that can make better use of BNF.

Figure1. Field experiment with stalk seeds of sugarcane inoculated with diazotrophic bacteria. Blue tubes are the bases of chambers installed to allow the collection of green house gases, including N_2O, emitted from soil and N fertilizer. Gas sampling in the field

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Six experiments have been set up in the field and most results will be obtained after harvesting. One of the difficulties to assess whether the N in the plant came from fertilizer or BNF is that most of the nutrient taken up actually comes from the soil where it is continuously recycled. Besides, sugarcane plants in the field have already a natural population of diazotrophic bacteria that may overshadow the action of inoculated bacteria strains. Therefore, long term observations were planed for the accounting of partial inputs and outputs of N in the field, which includes the monitoring of plant yields and of soil N stocks in situations where zero or high N fertilizer inputs are used, so as to infer about any sizeable contribution of N fixation from the atmosphere. In addition the δ15N technique is being used to assess BNF by analyzing the isotopic composition of N species in sugarcane plants grown under field conditions.

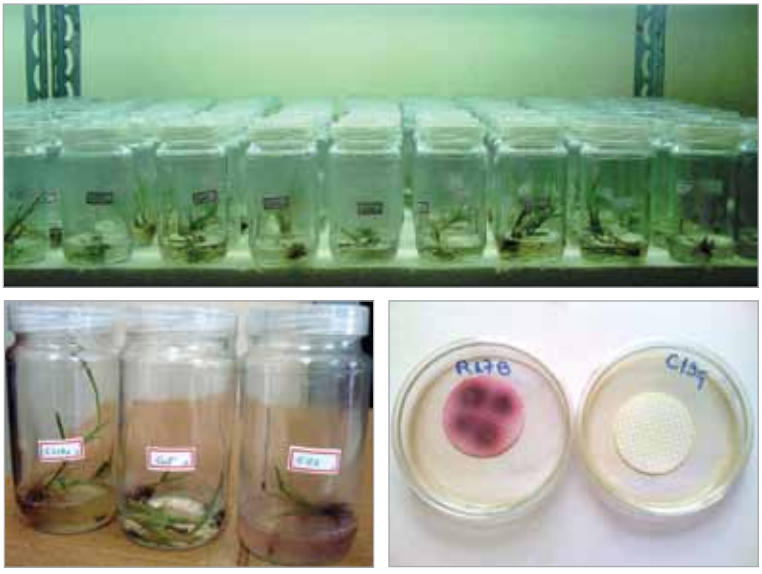

Figure 2. Above: micropropagated sugarcane plants inoculated with isolates of diazotrophic bacteria. Below left: diazotroph isolates growing in substrate containing sugarcane plants; Right: Isolate R178 showing indole production associated with plant growth promoting characteristics

Traits of different sugarcane genotypes associated with N_2 fixation will be characterized with molecular biology. *In vitro* tests are being carried out with several sugarcane varieties inoculated with the strain PAL-5 to evaluate their potential for BNF. At the same time the expression of genes involved with BNF and with the plant-bacteria interaction is being studied with the purpose of incorporating these characteristics in the sugarcane breeding program.

In order to broaden the community of known endophytic bacteria new isolations are being carried out. So far 160 isolates were obtained in one irrigated and one rain-fed experiment, from roots and stalks of varieties belonging to the breeding programs of Agronomic Institute, Ridesa, and Copersucar. Root colonization by diazotrophic bacteria was highly stimulated under irrigated conditions.

Eighty four percent of the isolates stood out as *in vitro* N_2 fixing organisms whereas 60% showed significant indole production. This latter characteristic is associated with phytohormone production that stimulate plant growth and development, which is another form of action of some of the diazotrophic bacteria. All isolates are being tested as to their capacity to promote growth of micropropagated sugarcane plants and, eventually to be used as inoculants.

If BNF can be managed in order to reduce the use of N fertilizer, either by inoculating plants in the field, or by learning the conditions that could favor N_2 fixation or still by breeding plants that can best benefit from BNF, the impact for bionergy production from sugarcane will be of great economic and environmental significance.

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