The importance of biological nitrogen fixation to biomass production from *Pennisetum purpureum*. A renewable energy source

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Nowadays there is a strong interest to find efficient renewable energy sources as alternatives to the use of gasoline. The burning of fossil fuels keeps enriching the atmosphere with CO_2, which is one of the principal causes of increased global warming. For this reason and taking in consideration that the world oil reserves are finite, it becomes increasingly evident that one of the best's alternatives could be the use of solar energy captured by photosynthesis of plants, known as biomass energy. It is important to remark also that the CO_2 released from the biomass burning is part of the natural process of recycling of the C fixed by photosynthesis.

Biomass production is highly dependent on the soil fertility, where the soil N availability is one of the most important factors that affect the biomass production, especially in the less fertile soils of developing countries, and for this reason the use of N-fertiliser is very common. As fertiliser, especially N, has a very high input from fossil fuels it is necessary to optimise its use in order to always produce biomass with a very positive energy balance. Nowadays Brazil has the largest liquid biofuel production program in the world based on the sugar cane crop, the use of N-fertiliser in this crop is very low (mean of 60 kg N ha^{-1}) compared to other countries where farmers to obtain similar yields (60 to 100 Mg ha^{-1}) need to apply at least the double quantity nitrogen. The contribution of the associative biological nitrogen fixation (BNF) to the plant nitrogen nutrition is being considered as the principal mechanism to the low response of this crop to the N-fertiliser application, contributing in this form to the high energy balance of the ethanol production from sugar cane of more than 9 [1, 2, 3, 4].

In the same manner, another crop that can produce large quantities of biomass is the fast-growing C4 grass *Pennisetum purpureum* commonly known as elephant grass that grows very well in the semi-humid or humid tropics. This crop has a great number of genotypes and several of these that are highly enriched with protein and respond almost linearly to N-fertiliser application are being used as forage for dairy cattle. However, this crop has also some genotypes of low forage quality that grow and produce very well in poor soils and do not demand large quantities of N-fertiliser. Thus these *Pennisetum* genotypes of high biomass yield have great potential to be used as a energy crop, substituting firewood and a fuel and also to make charcoal for iron production. Based on this hypothesis, at the research Centre of Embrapa Agrobiologia a research project was started recently to select *Pennisetum* genotypes for high biomass yields of high C:N ratio (maximum C content for minimum N input) and for their possible capability to obtain N from BNF. For this, two experiments were carried out in the field. The first one had the objective to screen *Pennisetum* genotypes for high biomass yield and BNF efficiency. This experiment is being developed in concrete cylinders of 0.6 m diameter and 1 m depth filled with samples of a poor clayey Hapludult soil labelled with ^{15}N. In this cylinders fourteen *Pennisetum* genotypes with eight replications were planted in May 1995. The contribution of BNF to plant N nutrition is being evaluated by the ^{15}N isotope dilution technique [5]. The populations of diazotrophic bacteria in washed roots and in the aerial parts of the plants were also evaluated [6]. Every year all cylinders receive appropriate fertilisation but no N and the aerial part of the plants are being harvested twice a year. After a suitable cutting height for the crop had been selected, there were large differences in dry matter production, N accumulation and ^{15}N enrichment, and here we present some preliminary data. Some genotypes showed much higher dry-matter production than others reaching the equivalent of 81 Mg ha^{-1} $year^{-1}$ (Gramafante or Cameroon Piracicaba) compared to others such as Mott which only produced a mean annual of 15 Mg. Total N accumulation by plants also varied widely between varieties ranging from the equivalent of 393 kg N ha^{-1} year^{-1} for Gramafante and between 240 and 270 kg N ha^{-1} year^{-1} for the varieties Mineiro, Cameroon-Piracicaba, Piracicaba P241 and Sem Pelo, to only 75 kg N ha^{-1} for the variety Roxo. As the genotype Roxo was one of the lowest producing plants, this was used as reference to quantify the BNF contribution, and these values ranged from between 14 to 36% of plant N derived from BNF at the ninth harvest and confirmed one year later.

Based on the promising results of the cylinders experiment, three of the most productive *Pennisetum* varieties (Gramafante, Cameroon Piracicaba, Merker x 239DA-2), and the variety Roxo (as a low productive control) with four replications were planted (May 2000) into 4 replicated plots of 3 x 5 m in a sandy low fertile soil (Arenic Hapludult). This experiment is being developed at the Embrapa Agrobiologia experimental station. At planting the soil was properly fertilised with macro and micronutrients but no N-fertiliser was applied. The contribution of the BNF to the plant N nutrition was measured by the ^{15}N natural abundance technique [7]. Under this condition, similar results to the cylinder experiment were observed, the dry matter production of the three most efficient genotypes varied around of 60 Mg ha^{-1} harvest^{-1} and accumulated up to 400 kg N ha^{-1} in seven months. Using again the genotype Roxo as reference plant, the contribution of BNF in these genotypes varied from 25 to 40%, equivalent to 106 and 165 kg N ha^{-1} harvest^{-1}, respectively. As in both experiment high number of diazotrophic bacteria, especially of the genus *Herbaspirillum* [8] were found associated with shoots and roots of all *Pennisetum* genotypes, it is probably that the values of BNF associated to this crop could be higher, and this could better explain the high values of N accumulated by this *Pennisetum* genotypes growing in this poor soil.

Conclusion

The *Pennisetum purpureum* genotypes, Gramafante, Cameroon Piracicaba and Merker x 239DA-2 showed very high biomass yield in poor soils and have a great potential to be used as a renewable energy source. The BNF associated to these genotypes is contributing significantly to replace N-fertiliser, improving the positive energy balance.

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