Biodiversity, Sustainability and Drug Development

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Which Class of Antihypertensive Agents Arose from the Tropical Rain Forests of South America?

ACE inhibitors. In the 1960s, Ferreira discovered that venoms of the South American pit viper (Bothrops jararaca) contained peptides that inhibited angiotensin converting enzyme. The molecular structure of these natural peptides provided the starting point for the design of the commercial ACE inhibitors used today.

This was a Today's Question of the Medscape website. The answer conveys right and wrong notions. The term "rain forests" immediately evoke the idea of a forest around the equator like the Amazon forest. In fact it is little realised that the Mata Atlântica is much rich in biodiversity than the Amazon forest, which it is now almost destroyed due to the expansion of the agriculture and urban areas. The venom used in those pioneer experiments was original of a family of snakes abundant in the Mata Atlântica.

It is correct, however, to call attention for the fact that a great majority of synthetic medicaments were based in prototype molecules extracted from natural product, like snake venom or a plant extract. The crucial moment for the development of a drug is the discovery of the prototype. This prototype has the expected activity of the drug, but usually requires further development because of its low potency, short biological life, high price, difficult synthesis, undesirable side effects, etc.

The text gives a wrong notion, because it implies linearity between the discovery of the prototype and its use as an inhibitor of the angiotensin-converting enzyme. Drugs are generally developed by the industry. Prototypes nevertheless can be discovered in the Academia and in general there is a long way to be made for the drug to reach the hands of the clinician.

At present, scientific knowledge is crucial for the development of new inventions, which guarantee industrial profit (1). However, the recognition of the academic contribution to the development of a new drug is, in general, very controversial.

"From *Bothrops jararaca* bradykinin potentiating peptides to angiotensin converting enzyme inhibitors" is a story which illustrates this point. Scientists who were doing non-task-oriented research, in academia, hospitals, and other non-industrial institutions produced the basic scientific knowledge up to the drug-prototype of the converting enzyme inhibitors. In effect, the discovery of the rennin-angiotensin and bradykinin systems and their possible contribution to cardiovascular homeostasis was pioneered by academic scientists such as Tiegersted and Bergman in Sweden, Werle and his collaborators Kraut and Frey in Germanyh, Braun-Menéndez in Argentina, Gold, Page and Helmer in the U.S., and Rocha e Silva in Brazil. Our own contribution to the development of the converting enzymes was mainly made at the University of São Paulo, Brazil. Progress, however, was only possible with the collaboration of several other scientists, such as L.J. Greene and J.M. Stewart in the USA, J. R. Vane and Y.S. Backle in England, and E.M. Krieger in Brazil (see 2).

When we discovered the bradykinin potentiating peptides in the venom of a Brazilian snake, *Bothrops jararaca,* we had no idea that the enzyme responsible for the major inactivation of bradykinin in the body was the same as that which converted the inactive e angiotensin I to the active hypertensive molecule angiotensin II.

Nonetheless, the bradykinin potentiating peptides were instrumental in this demonstration (see Ref. 2).

Our main contribution to the area can be summarised as follows: A) the demonstration of the inhibition of bradykinin destruction by metal chelating agents (3); B) the discovery of the bradykinin potentiating peptides in the venom of the Brazilian snake *Bothrops jararaca* and the demonstration of its pharmacological profile (4-7); C) the use of the bradykinin potentiating factor (BPF) as a methodological tool to detect the participation of bradykinin in physiopathological processes (8,9); D) the isolation of nine peptides possessing bradykinin demonstration of parallelism between bradykinin potentiating activity and inhibition of angiotensin I conversion (10,11); E) the elucidation of the structure, synthesis and definition of the pharmacological profile of the smallest active *Bothrops* peptide, PCA-Lys-Trp-Ala-Pro, BPP_{5a} F) the first demonstration that the synthetic pentapeptide was effective in controlling blood pressure was in angiotensin-dependent models of hypertension (15). Later, one of the *Bothrops* venom peptides synthesised by the Squibb group was successful in testing the hypothesis that converting enzyme inhibitors were effective in lowering blood pressure in hypertensive patients (16). It is intriguing that, at this point in the sooty, the family of bradykinin potentiating peptides (BPP) was rather unethically renamed "angiotensin converting enzyme inhibitors" (17), probable because of marketing objectives. The awareness that the converting enzyme could be inhibited by metal chelating agents (3), that BPP_{5A} was split by the converting enzyme in the carboxyterminal amino acids, together with the new concept of making non-peptidic drugs from peptides (18), allowed Squibb reconcept of making non-peptidic drugs from peptides, in particular BPP_{5a} , could be considered as the drug-prototype since they were instrumental for the development of this first member of a new class of antihypertensive drugs. The clinical success of Captopril and the other "me too" angiotensin converting enzyme inhibitors popularised the concept that the only mode of action of the renin-angiotensin system. However, mounting evidence has been obtained over the last few years that the beneficial effects of this group of drugs on the reduction of cardiac insufficiency and myocardial infarct lesions is mainly due their bradykinin potentiating activity (20-24).

Thus it is not unfair to say that most of the relevant basic knowledge used ion the invention of Captopril was the result of non-task-oriented academic research. We know very well that there is no typical inventor since, in the process of inventing, developmental scientists frequently make scientific discoveries. But it is well recognised that drug development is heavily supported intellectually by academic research (1).

The question to be asked is: why doesn't new scientific knowledge have intellectual property rights?

As academic scientific discoveries are published (which does not happen to a great deal of industrial scientific discoveries), they become of public domain. There is a tacit international consensus that public domain prevents the authors from participating in the patent rights of any invention resulting from their scientific discoveries. This may be acceptable for developed countries, whose industries are capable of transforming the new scientific discoveries into inventions. For the third world, however, the system is unfair, since third world countries lack industries strong enough to develop new products. Even in developed countries, however, scientists working in universities are now under fire and their survival is frequently linked to their industrial development or task-oriented projects. Alternatively, scientists now frequently withhold their scientific findings and offer them to the industry before any publication. This pattern of behaviour will eventually sterilise the scientific creativity, which used to flourish in the intellectual freedom of academia.

At present, at the University of São Paulo, in Brazil, we are repeating the BPF story by developing prototypes of new analgesics based on new knowledge regarding the physiopathology of inflammatory pain which has been discovered in our laboratories. It is obvious that I will get recognition from my academic and industrial colleagues for those discoveries. But without recognition from of the intellectual property rights, my country and university will never profit from the investment that was made in my education and research. I am hoping that my colleagues intellectual understand the importance of fighting for the international recognition of the intellectual property rights for scientific discoveries in order to participate in a fraction of the value of the patents. This recognition will certainly protect scientific research in the university not only in undeveloped countries but also around the world, which is now on the brink of collapse.

Biodiversity is a very broad concept involving all nature's living entities and basic principles of its preservation and protection including principles of sustainability, intellectual property rights and transfer of new technology for the native population etc.

Degradation of the ecosystem certainly was not due to the research and production of new drugs. Drugs like rutine and pilocarpine, which are still extracted from plants, are produced in dedicated farms. The research of new drugs profit immensely of the biological diversity and the knowledge of its native population. In the year of 1988,for example, among the twenty drugs introduced in USA the market, seventeen had their prototype directly originated from plants.

Brazil like may other countries have its biodiversity threaten by globalisation. We stand in the international market as cheap exporters of our flora, soil and our ethnobotanic and **ethnopharmacological** knowledge of our biodiversity. In fact to the native knowledge of the medicinal use of the plants added those of the African slaves and Europeans. Brazilian laws that "protect" our biodiversity are frequently meaningless either because the rain forest belongs to several countries or the sampling of materials for drug development can be made without social noise and in general with the cooperation of a Brazilian research associate.

We have recently evaluated the effort that the Brazilian made in the investigation of medicinal plants. In part, this effort was stimulated by the controversial idea of substitution of standard therapy by cheaper herbal therapy. But there was also the sensible idea of determining the active principle and the toxicological profile of the plants in popular use.

Most of these studies, however, took us no where, but allowed in the last thirty years the formation of a great number of postgraduate students and a few qualified clinical and experimental laboratories. Brazil to profit from our immense biodiversity must organise a strategic plan for drug development in charge of the coordination of national and international groups interested in natural product research. A detailed description of a strategic planning was described elsewhere.

Finally, we would like to point out that there is a general recognition of the importance of biodiversity for the development of drugs. However, we also recognise that the total sustainability of biodiversity is improbable with the presence of men in forest regions. Men actively adequate the environment for their security. They actively decimate aggressive or predatory animals and apparently inutile plants, which entails the destruction of their ecological partners. Scientists also know that they can only invent drugs with the screening tests available to them. Future screening tests will be developed with the new understanding of diseases or pathological processes. It is obvious that we cannot use now the screening tests of the future. Thus, if we do want to preserve biodiversity for future research and for the discovery of new drugs, we cannot just organise its sustainability. It is crucial to preserve a certain number of sanctuaries, inaccessible to any kind of exploitation and representative of the biodiversity of the various ecological regions around the world.

References

1. Conroe, J. H. & Dripps, R. R. (1976). Scientific basis for the support biomedical science. *Science*, 192: 105 –111.

2. Ferreira, S. H. (1985). History of the development of inhibitors of angiotensin I conversion. *Drugs,* 30: 1-5.

3. Ferreira, S. H. & Rocha e Silva, M (1962). Potentiation of bradykinin by dimercaptopropanol (BAL) and other inhibitors of its destroying enzyme in plasma. *Biochemical Pharmacology*, 11: 1123-1128.

4. Ferreira, S. H. (1965). A bradykinin-potentiating factor (BPF) present in the venom of *Bothrops jararaca. British Journal of Pharmacology*, 24: 163-169.

5. Ferreira, S. H. (1966). Bradykinin-potentiating factor. In: Erdös EG (Editor), *Hypotensive Peptides.* Springer-Verlag, New York, 356-367.

6. Ferreira, S. H. & Rocha e Silva, M (1965). Potentiation of bradykinin and eledoisin by BPF (Bradykinin Potentiating Factor) from *Bothrops Jararaca* venom. *Experimentia*, 21: 347-351.

7. Amorim, D. S.; Ferreira, S. H.; Manço, J. C.; Tanaka, A.; Sader, A. A. & Cardoso, S. S. (1967). Potentiation of circulatory effects of bradykinin by a factor contained in the *Bothrops jararaca* venom. *Cardiologia*, 50: 23-32.

8. Ferreira, S. H. & Smaje, L. H. (1976). Bradykinin and functional vasodilatation in the salivary gland. *British Journal of Pharmacology*, 58: 201-209.

9. Rocha e Silva, M.; Reis, M. L. & Ferreira, S. H. (1967). Release of kinins from fresh plasma under varying experimental conditions. *Biochemical Pharmacology*, 16: 1665-1676.

10. Ferreira, S. H., Bartelt, D. C. & Green, L. J. (1970). Isolation of bradykinin-potentiating peptides from *Bothrops jararaca* venom. *Biochemistry*, 9: 2583-2593.

11. Ferreira, S. H.; Greene, L. J.; Alabaster, V. A., Bakhle, Y. S. & Vane, J. R. (1970). Activity of various fractions of bradykinin potentiating factor angiotensin I converting enzyme. *Nature*, 225: 379-380.

12. Stewart, J.M., Ferreira, S. H. & Greene, L. J. (1971). Bradykinin potentiating peptide PCA-Lys-TRP-Ala-Pro. An inhibitor of the pulmonary inactivation of bradykinin and conversion of angiotensin I to II. *Biochemical Pharmacology,* 20: 1557-1567.

13. Greene, L. J.; Stewart, J. M. & Ferreira, S. H. H (1970). Bradykinin-potentiating peptides from the venom of *Bothrops jararaca. In*: Sicuteri, F.; Rocha e Silva, M. & Back, N. (Editors), Bradykinin and Related Kinins: Cardiovascular Biochemical and Neural Actions. Plenum Press, New York, London, 81-87.

14. Greene, L. J., Camargo, A. C. M.; Krieger, E. M.; Stewart, J. M. & Ferreira, S. H. (1972). Inhibition of the conversion of angiotensin I to II and potentiation of bradykinin by small peptides present in *Bothrops jararaca* venom. *Circulation Research*, XXX & XXXI: 62-71.

15. Krieger, E. M., Salgado, H. C.; Assan, C. J., Greene, L. J. & Ferreira, S. H. (1971). Potential screening test for detection of overactivity of **renin**-angiotensin system. *Lancet*, 1: 269-271.

16. Gavras, H.; Brunner, H. R.; Laragh, J.; Sealey, J. E.; Gravas, I & Vukovich, R. A. (1974). An angiotensin converting-enzyme inhibitor to identify and treat vasoconstrictor and **colume** factors in hypertensive patients. *New England Journal of Medicine*, 291: 817-821.

17. Ondetti, M. A.; Williams, N. J.; Sabo, E. F.; Plusced, J.; Weaver, E. R. & Kocy, O. (1971). Angiotensin-converting enzyme inhibitors from the venom of *Bothrops jararaca.* Isolation, elucidation of structure and synthesis. *Biochemistry*, 10: 4033-4039.

18. Byers, L. D. & Wolfenden, R. (1972). A potent reversible inhibitor of carboxypeptidase. *Journal of Biological Chemistry*, 247: 606-608.

19. Cushman, D. W.; Cheung, H.S.; Sabo, E. F. & Ondetti, M. A. (1977). Design of potent competitive inhibitors of angiotensin-converting enzyme carboxyalkanoyl and mercaptoalkanoyl amino acids. *Biochemistry*, 16: 5484-5491.

20. Linz, W.; Albus, U. & Schölkens, B. A. (1989). Effects of endothelin on isolated ischaemic rat hearts during ramiprilat, bradykinin and indomethacin perfusion. *Journal of Hypertension*, 7: s310-s311.

21. Schölkens, B. A.; Ling, W. & Konig, W. (1988). Effects of the angiotensin converting enzyme inhibitor ramipril in isolated ischemic rat heart are abolished by a bradykinin antagonist. *Journal of Hypertension*, 6: s525-528.

22. Lins, W.; Wiener, G. & Schökens, B. [a] (1994). Cardioprotective actions of bradykinin in myocardial ischemia and left ventricular hypertrophy. *Brazilian Journal of Medical and Biological Research*, 27: 1949-1954.

23. Hecker, M.; Pörsti, I. & Busse, R. (1994). Mechanisms involved in the angiotensin II-independent hypotensive action of ACE inhibitors. *Brazilian Journal of Medical and Biological Research*, 27: 1917-1921.

24. Nolly, H.; Damiani, M. T. & Miatello, R. (1994). Vascular-derived kinins and local control of vascular tone. *Brazilian Journal of Medical and Biological Research*, 27:1995-2011.

Sustainable Agriculture in the Tropics

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Unfortunately there is no a vaccine against hunger. People need to be adequately fed every day, hence the importance of a well developed agriculture that rationally utilizes available natural resources together with the technological improvements resulting from scientific research

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I - The Struggle to Survive

For most of the 1.5 million years of the **estimated** existence of mankind on this planet, human beings had to face all sort of adversities, competing with animals more apt to live in a hostile environment. A major concern was always an adequate food supply. Hunting and collecting plants provided food, although irregularly. Several times the human species came close to extinction as pointed out by 1970 Nobel Peace Prize laureate, Norman Borlaug , the father of the Green Revolution. During that long time, acting essentially as a hunter, there was little social progress. The Sole concern was survival. No significant increase in population took place, with a tendency to stabilize at around ten million individuals (Borlaug 1972).

About 10,000 years ago plants began to be cultivated. It is believed that this initiative was pioneered by women, who are less apt at hunting and having to take care of domestic tasks, had more opportunities to observe plants, to develop knowledge on their properties and eventually on their reproduction. Agriculture is a quite recent activity in the history of humankind. This can be easily visualized using a scale of 365 days to represent 1.5 million years, where man appears on January 1st and agriculture is initiated at four thirty P. M. on December 29.It is of interest to point out that agriculture was invented at least twice independently, in the Old and in the New World. Both types differ in many respects, such as animal vs. human work, selection for uniformity in the Old World vs. selection for variability in colors and shapes in the New World, as can be appreciated in many crops such as maize, beans, squashes and so on. Besides, inhabitants of the New World never discovered the wheel as a means of transportation, and even though they succeeded in developing some animal husbandry in North America, no animal domestication to work in agriculture took place. In this short period in historical perspective, agriculture had a significant development as can be seen on Table 1. A primitive husbandry improved by a factor of ten the results of hunting. But, no doubt, subsequent advances in agriculture were much more spectacular. Subsequent improvements in agriculture made possible the development of communities and later on of cities. Also fewer people were necessary to produce food, so that many could dedicate their time to other activities such as commerce, arts, politics, waging war , religion and so on.

In spite of the progress achieved, there has been a recurrent concern on the prospect of hunger due to limitation of food production. The catastrophic prediction made by the Rev. Robert Malthus in 1798 is well known, stating that hunger would be inevitable, for while the population increases hardly in a geometric progression, food production increases scarcely in an arithmetic progression. Such “prophecies” have been repeated more recently by Paul Ehrlich in his book “The Population Bomb” published in 1966: a “prediction” was made to the extent that in the sixties and seventies hundreds of millions would die of starvation. Thanks, however, to technological progress applied to agriculture such disasters did not occur, although many still believe that such predictions have been merely postponed.

Food is the most pressing need for human beings. Besides improvement in agriculture, food availability depends on social and economical conditions of the communities. In 1913 the Rockfeller Foundation was created with the goal to improve the quality of life. The first 20 year emphasis was on health. Then an assessment of the program reached the conclusion that the five basic needs for a satisfactory quality of life are Food, Health, Shelter, Education and Opportunity, in that order:

II - Plant Domestication

Changes due to domestication

The enormous plant diversity has not been evenly utilized for food production. From the known 350.000 species, man employed during all his history less than 3000 and today about 300 are cultivated. The fifteen most important, contributing with more than 90% of all food production in the world are rice, wheat, maize, soybean, sorghum, barley, sugarcane, sugar beet, beans, peanut, potato, sweet potato, cassava, coconut and banana. These species are the result both of the selection among many originally used as well as of the selection within each species to increase its efficiency in providing adequate quantity and quality of food.

Many characteristics were changed in the process of domestication from the original wild species. The most evident are the following:

a) Loss of natural seed dispersion, so that the grains remain attached to the spike, helping the harvest.

b) Loss of seed dormancy, so that all seeds germinate uniformly.

c) Change from alogamous to autogamous reproduction in many species.

d) Change from perennial to annual life cycle in several species, which increases yielding ability per unit of land

e) Change from dioecism (male and female plants) to monoecism or hermaphrodites (both sexes in the same plant).

f) Increase in the size of fruits, grains and general yielding ability, besides several characters such as quality, flavor, and so on.

All these changes, including domestication, are the result of empirical selection carried out by rural communities during hundreds or thousand years, indicating the skillfullness of ancient cultivators and also the great amount of genetic variability within species. Also, it should be emphasized that all present day cultivated plants were domesticated by ancient people. Modern man received from their predecessors all domesticated plants showing the mentioned changes. By applying the knowledge obtained especially in the present century, man continues the improvement, achieving highly significant gains in food production.

Vulnerability of domesticated plants

Although plant domestication assured the survival of human species, it also raised some unexpected problems. The most serious are represented by the vulnerability of the cultivated plants to diseases caused by microorganisms, especially fungi and bacteria. When in the wilderness, plants were dispersed in the environment mixed to other species, which conferred them some degree of protection. Although some could be infected, most escaped and remained healthy. Besides, a great genetic variability of the wild species was an additional asset favouring the occurrence of genetically resistant plants. Under domestication artificial selection during many generations increased uniformity, resulting in a reduction of genetic variability. Also, plants became cultivated in more compact and denser populations, favouring still more the spread of diseases.

Since biblical time reports can be found on disasters in food production due to diseases. In ancient Rome, Plinius considers “the wheat rust the greatest curse for crops”. Perhaps the most dramatic example was the potato blight appearing from 1830 to 1840 in Western Europe and Northeastern USA, reaching catastrophic proportions on 1845 in Ireland. Being the staple food, the blight was so violent that about one million people died of starvation and another million emigrated to US. Later on the fungus called *Phytophtera infestants* (from the Greek Phyto = plant and phthera = destructor) was identified as responsible for the disease. This disease also affected the German crops during the First World War, what might have contributed to abreviate its end.

III - Tropical vs. Temperate Climate Agriculture

Regions of the Northern Hemisphere most of them belonging to the First World, have a more temperate climate and are more developed than most tropical countries on the Southern Hemisphere. Furthermore, frequently, there is a tendency to make comparisons with the most developed nations. Regarding agriculture, this would imply compairing temperate to tropical environments. This is highly inappropriate because the climatic differences between these two areas are markedly different, with the tropical regions being much more adverse regarding agriculture. Table 2 based on Brewbaker (1985) with some additional items (Paterniani 1990) summarizes the main characteristics of these two climates for maize production that can be applied also to other summer crops. Most of the characteristics are self-evident and need no further comment, except maybe to acknowledge the usually more adverse factors in the tropics. Unpredictable rainfall variation, certainly, is one of the most important constraint, as can be seen on figures 1 and 2 that compare monthly rainfall variations between a temperate location (Ames, Iowa, USA, 42° N, 93 ° W) and a sub-tropical place (Piracicaba, SP, Brazil, 22° S, 47° W) for a period of about 70 years. Some other items can also be highlighted to show the more adverse conditions for the tropics, such as the longer day length in temperate areas (15 to 16 hours of sunlight in summer) while in the tropics the summer has considerably shorter day length, i.e. about 13 hours. In this way, higher CO_2 fixation due to photosynthesis is the rule in temperate climates in relation to the tropics. Besides, shorter and cooler nights are more favourable in temperate areas, since less CO_2 is lost due to respiration, while the opposite is true in the tropics where longer and warmer nights prevail. Another important constraint refers to the soil: in temperate regions, the soils are usually less acid, more fertile, have a higher primary mineral reserve and more active clay, and are less apt to leaching and to erosion. Last but not least many of the technological developments that could improve tropical agriculture, although available, are not used due to poor social and economic conditions in most tropical countries or, in brief, due to lack of “sustainable” agricultural policy in most cases.

Plants are dependent on climate, so that species or varieties are adapted to specific climatic environments . This renders even more inappropriate the comparison between tropical agriculture with the temperate one, since plants need to be adapted and improved to specific conditions, which is not the case regarding industrial appliances for example. The technology must be developed in the area where it is going to be used. Agricultural practices, plant nutrition, fertilizing, plant breeding, disease and insect control, all these items need to be developed in the tropics, for most of the technology adequate in temperate areas are of no use in the tropics. In agriculture, transfer of technology is usually an inadequate option. It is quite obvious that the assessment of the agricultural efficiency should be done between regions of similar climatic conditions. In this regard, Brazil has been able to carry out important basic and applied research, that resulted in a well developed and reasonably efficient agriculture, superior to most other tropical countries. Many of these technical achievements are available for immediate employment or adaptation to other tropical countries a high probability of promising results.

IV - Improved Technology in Modern Agriculture

Although some know how relative to plant cultivation, such as notions on mineral nutrition, sexual reproduction and other agricultural techniques were already available in the 19th century, agriculture as an applied science is a development of the 20th century. Being an applied science, it is only natural that investigators in agriculture tried to use as much as possible the advances in basic science to improve agriculture efficiency. The following scientific landmarks are pertinent:

a) The start of the fertilizer industry as early as 1840 with the production of simple superphosphate by Lawes in England, shortly after Liebig in Germany established that plants are fed by air, water and a handful of minerals.

b) Mendelian inheritance, due to the work of Mendel in 1865, and its rediscovery in 1900 by De Vries, Correns and Tschermack.

c) Heterosis or hybrid vigor in maize (East 1908 and Shull 1909), later on extended to other crops, which might be the greatest contribution of the present century to agriculture.

d) The contribution of Thomas Hunt Morgan, around 1910, showing the role of chromosomes in inheritance.

e) Genetic basis of complex characters, called quantitative (Nilsson – Ehle 1908 and Fisher 1918), which led to the development of new and more efficient methods of plant breeding.

f) The development of statistical methods by Fisher in 1917 (Fisher 1936), that led to the development of experimental designs to achieve greater precision in field work evaluations.

g) The effect of ionizing radiations for the production of mutant genes by J. H. Muller around 1930.

h) The finding that the resistance and the susceptibility of plants to diseases caused by fungi are controlled by genes and, in sequence, Flor´s theory (Flor 1955) that for every gene for virulence in a fungus, there is a corresponding gene for resistance in the host plant.

i) Several methods for plant breeding, such as methods for obtaining evaluating inbred lines for hybrid production, recurrent selection schemes (both intra and inter-population) and methods for improvement of autogamous and asexual reproduction species.

j) A series of discoveries related to the genetic material (DNA) that led to Molecular Genetics and Genetic Engineering with the production of transgenic plants.

k) The development of the concept of Integrated Pest Management (IPM), where pests are controlled using a combination of techniques such as: chemical, genetic resistance, environmental control, cultural practices and biological control.

l) A number of agricultural practices, resulting from improvements on mechanization and equipment, also played an important role to increase efficiency in agriculture, such as better irrigation systems like "Central Pivot" no-till farming and equipments to improve land preparations, plant cultivation, protection and harvesting.

There has been world-wide increase in the productivity per unit of land, thanks to the use of the improved available technology. It is currently estimated that about 18 million km^2, an area equivalent to South America, is cultivated throughout the world. If crop yields would be at the level of 1950, there would be the need to plow about 48 million km^2 (Avery 1994). Thanks to modern technology developed countries are increasingly going to "high-yield farming", a combination of techniques to provide higher yields per unit of land. Since 1968 Sweden shifted more than 5 million hectares back into forest, without decreasing crop productivity (FAO 1969, 1991), while Chile, with no increase in cropland, has been able to feed a population growing at 1.7 % annually and at the same to time expand the export of its fruits and vegetables. Ecuador with yields not rising due to low-yield agriculture, is cutting its forests and expanding its cropland at a rate of about 2% annually (Avery, 1994).

Many reports indicate a need to increase food and fiber production to satisfy the needs of a growing world population. Cultivable land per capita is being reduced significantly, from near 1 ha in 1960 to 0.5 in 2000 and 0.30 in 2040 (Krattiger 1998). Although not all countries are in the same situation, for many countries like Brazil still have plenty of land to be cropped, the rational use of available techniques to improve efficiency in agriculture should help to protect land devoted to wild life, helping in this way to improve the environment, an end of the sustainable approach.

Although the techniques for high–yield farming are widely known, they are not employed everywhere, specially by poorer countries, in view of their socio-economic conditions.

V - Some examples of Brazilian Agriculture

General aspects

Considering the constraints of the tropical environment, together with other limiting factors of infrastructure, it is significant that currently Brazilian agriculture lies at the front in comparison to other tropical areas of the world with respect to efficiency and productivity. Besides this advanced position, Brazil is experiencing a continuous progress in production per unit of land. In the period 1970/1995 productivity of 16 important crops was doubled, thereby "saving" about 50 million hectares of land from cultivation. The following factors are responsible for this condition: the development of new improved varieties of the most important crops; techniques of integrated pest management (IPM), where biological control plays an important role especially reducing the use of agrochemicals; the conquest of the "cerrados" (savannas) a type of soil considered several years ago inappropriate for agriculture. In addition, techniques for soil conservation, no-till farming, more efficient use of fertilizers and enhanced nitrogen fixation by strains of Rhizobium sp. have also played their role.

No-till farming, clean air and clean water, is now used in about 10 million hectares in the South, West and Center of this country.

Many tropical countries have benefited with the improvements achieved by Brazilian agriculture, including the use of improved varieties. Much more can still be adapted abroad, for instance, the improvement of savanna like soils.

Agricultural research in Brazil has been carried out both by official institutions and by the private sector. Official institutions, corresponding to public universities, state institutes and the Brazilian Enterprise of Agricultural Research (EMBRAPA), contributed to basic and applied research. These institutions receive support from several financing government institutions as National Research Council (CNPq), Financing of Studies and Research (FINEP), Coordination to Improve University Professors (CAPES) and also State Foundations for the Advancement of Science. Important contributions from the private sector resulted in improved varieties, like hybrid maize, vegetables and other crops, and in the area of fertilizers, agrochemicals and mechanical equipments.

Genetic Improvement

Nothing is more essential for agriculture than the seed. Seeds here are understood in the broad sense, comprising the true botanical seeds, as well as any propagating material used commercially such as vegetable parts employed for assexual propagation. One must realize that the value of a seed is a function of its genetic potential. Agricultural productivity of good quality and quantity is achieved only by a proper combination of genetic quality with the more advanced agricultural practices. Some examples of genetic improvement are provided below.

Coffee: Coffee breeding was initiated in Brazil in 1933 at the Instituto Agronômico de Campinas, São Paulo. Fundamental basic research on taxonomy, cytogenetics and biology of flowering paved the way to improve coffee breeding. Table 3 shows the improvement achieved by selecting progenies of the Mundo Novo variety over previous ones. Subsequently new varieties were obtained with higher yield and important characteristics like resistance to rust (*Hemileya vastatrix*).

Maize. Maize is the major crop in Brazil with respect to cultivation area. Several official institutions have devoted a great amount of effort to maize research, both basic and applied. Studies on germplasm characterization, utilization and preservation have resulted in substantial knowledge of the genetic potential of races and varieties, both local and introduced. New breeding schemes, especially related to population improvement, have been developed employing methods of recurrent selection and reciprocal recurrent selection. Improved varieties have been obtained that were used per se by farmers and also as basic material to develop better inbred lines to produce superior hybrids. Table 4 gives the results of the evaluations of genetic improvement for grain or ear weight conducted by several investigators. It can be seen that the yearly progress is continuous. Besides grain yield, substantial improvements have been obtained in agronomic characters, such as reduction of plant and ear height, resistance to lodging, resistance to diseases, among others.

In the last 15 years, farmers started planting maize off season, sowing around February. This started in the State of Parana State, in substitution to wheat. This type of cultivation gave satisfactory results, so that, nowadays maize is grown almost all year round. In consequence, new breeding programs had to be created do develop cultivars, especially hybrids, adapted to the new season that includes winter.

Soybean. Until 1970 Brazil was planting very little soybean, representing only 2% of the world production. In 1998, due to the increasing economic importance and breeding programs, Brazilian soybean represents about 20% of the world production. Breeding programs were able to improve yields per unit area approaching those of the U. S. A. Although most soybeans are grown in Southern Brazil, genetic research developed varieties adapted to Central West and the North in Maranhão State. The progress achieved can be easily assessed, by the following figures: in 1961, grain yield was 1, 127 kg/ha, in 1980, 1,727 kg/ha and in 1998, 2,367 kg/ha. This corresponds to an annual increase of 31.6 kg/ha/year or a gain of 1200 kg/ha in 38 years of research. Besides grain yield, improvement was achieved also for protein and oil content and quality, and resistance to diseases.

Bean. Bean (*Phaseolus vulgaris*) is the main staple food and source of protein for Brazilian people. In Northern States cowpea (*Vigna unguiculata*) is also quite popular. A variety of *P. vulgaris*, Carioca, is the most cultivated and has been selected to obtain new strains. Improvements both in cultural practices and in genetic gain have been observed. Evaluation along 20 years have shown a total gain of the order of 42.6 kg/ha/year with the genetic progress being of the order of 14.5 kg/ha/year. (Abreu et al. 1994)

Rice. Rice (*Oryza sativa*) is the most consumed food in Brazil, about 75 kg per capita per year. Three kinds of cultivation are used:

a) irrigated with controlled flooding; b) humid lowland without controlled irrigation and c) dryland. In dryland cultivation areas with low levels of water deficiency and adequate soil fertility and areas where low dry periods are frequent are used.

Rice breeding programs are underway by official institutions, both for irrigated and for dry land cultivation. Evaluations made by Soares and Ramalho (1993) and Rangel *et al.* (1996) have shown, for a period from 1974 to 1996, a genetic gain of 33 kg/ha/year for dry land and 44 kg/ha/year for irrigated rice.

Wheat. In Brazil *Triticum aestivum* is the most cultivated type of wheat, and in small scale *T. durum*. Some areas of triticale are also found. Even though Brazil imports most of the consumed wheat, programs for wheat improvement have been conducted with satisfactory success. From 1970 to 1996, a continuous trend in yield of the order of 38 kg/ha/year has been estimated, from which the genetic contribution is 17 kg/ha/year (Nedel 1994).

Temperate fruits. Several fruits of temperate climate are grown in Brazil, namely: apple, peach, nectarine, pear, plum, fig, strawberry and European nuts. Quite successful breeding programs have been conducted especially with apple, peach, pear and plum. Originally these species were not adapted to Brazilian climate, since they needed large periods of very low temperature to induce flowering. Genetic improvement was realized essentially through the evaluation of a great number of progenies, selecting the ones that require only a moderate period of cold to flower. In sequence, fruit quality like flavor, acidity, and other attributes are taken into consideration. Apple production in Brazil rose from 16,000 ton in 1977 to 495,000 ton in 1995. Productivity estimated from 1984 to 1995 showed a gain of 0.6 ton/ha/year. The country has become an exporter for very demanding markets. Varieties of the other mentioned temperate fruits adapted to Brazilian climate have been obtained, especially, peach, nectarine, pear and plum.

Eucalyptus: Introduced from Australia in the second decade of this century, eucalypt found a good environment in Brazil. It became the most important wood for general use. The area planted with eucalyptus rose from 700.000 ha in 1960 to 3.500.000 ha in 1998. Selection of genetic material has been done both by official and private institutions, resulting in significant improvement, as can be seen by a productivity of 20 m^3 /ha/year in 1960 that increased to 40 m^3/ha/year in 1998. About 50% of this gain is attributed to genetic improvement (Ferreira and Santos 1997). Liming phosphate fertilizers as well micronutrients, mainly boron and zinc, play a major role in the productivity of Eucalyptus and the quality of wood and fiber.

Vegetables. Until the forties most vegetables grown in Brazil were imported varieties that were more adapted to the local winter season. Almost no adequate adaptation existed for the summer, when vegetables become more important. Subsequent breeding programs were able to develop better adapted varieties both for the local winter and more importantly for summer. Main vegetables are: lettuce, carrot, brassicas (cabbage, cauliflower, broccoli), onion, eggplant, tomato and cucumber.

Citrus. Brazil is a leader in orange and the major exporter of orange juice. Several varieties are available and significant improvements were made regarding disease resistance. The production of nuclear clones to obtain stocks free from virus represented a significant advance in citrus production. Improvements of grafting techniques, including micrografting, further contributed to improve citrus production.

Integrated Pest Management

Pest control evolved to the point that a balance of different techniques has to be made: i.e. protection of environment, biological control, chemical control, genetic resistance of the plants, cultural practices. Depending on the pest intensity of infestation, local conditions and other factors, the most appropriate techniques or combination of techniques are employed.

In sugarcane the borer *Diatraea saccharalis* is the most important pest. In the past native flies have been used as parasitoid such as *Metagonistylum minense* and *Paratheresia claripalpis*. A parasite introduced from Trinidad Tobago *Cortesia flavipes* was shown to be much more efficient and is widely used . In the seventies with 10% infestation there was a loss of 100 million dollars annually. Today, thanks to the new parasites, the infestation is of the order of 2%, in spite of a much larger sugarcane area that is twice the as previous one (Macedo et al 1993).

In soybeans the worm *Anticarsia gemmatalis* is efficiently controlled by the *Baculovirus anticarsia*. In many other crops, like pastures, wheat, tomato, cotton, citrus, pests are being controlled using a combination of techniques representing Integrated Pest Management.

Plant Nutrition and Fertilizing

No adequate plant development can be obtained without an adequate supply of nutrients. A considerable improvement on plant nutrition and fertilization was responsible for most of the increase in productivity obtained for many crops in Brazil. It is proper to quote the later great P. R. Stout from Berkeley: "There is no miracle seed without fertilizer".

Malavolta (1999) presented a review of plant nutrition and adequate fertilization of the most important crops in Brazil, together with the needs to correct poor soils like the “cerrados”. The use of new areas to increase food and fiber production have been the general rule in many developing nations, including Brazil. This strategy represents a significant cost, even in money, compared to the rational use of fertilizers, besides the area that is saved.

The Conquest of the Cerrados

About 24% of Brazil is “Cerrado”, a soil type of savanna. Due to many deficiencies, “cerrados” were considered, several years ago, completely inappropriate for agriculture. Although there are different types of “cerrados” with regards to levels of nutritional defficiencies, the main constraints are high acidity, aluminum toxicity, and unavailability of most phosphorus for the plants. Thanks to basic studies to identify and understand the soil limitations, it has been possible to develop strategies to improve these areas, resulting in satisfactory productivity as can be seen on Tables 5 e 6.

The following point should be highlighted: the present generation inherited “cerrado” soils inappropriate for agriculture, since they were degraded by Nature. Due to scientific findings and proper management it delivering to the next generation a soil with good agricultural potential, which represents a significant sustainable approach.

Agriculture in the Amazon

Alvim (1999) presents an excellent report of the main factors related to possible and potential utilization of the Amazonian area in Brazil. Climate, vegetation and soil limitations are considered. Regarding the potential utilization for agriculture, Table 7 presents the condition for Continental Amazon. It can be seen that there is not a single environment typical of the Amazonian area, but different physical, biological, climatic and socio-economic aspects should be taken into consideration to indicate the most appropriate system for agricultural activity. The main systems for agricultural utilization of the Amazonian region is presented by Alvim (1999), identifying the following: perennial crops, forestry, pasture and annual crops. All these systems, if employed in the appropriated areas and using adequate techniques are quite sustainable.

VI - Conclusion

There are a number of agricultural systems, activities and managements. Scientific research evolved to the point where a substantial productivity can be obtained on various environments. High technology in agriculture should not be viewed as something against nature but, once properly applied, as a provider for enough food and fiber without the continuous need to use additional areas and clearing of forests. The examples reported show the benefits of scientific research and its applications to improve productivity, and at the same time providing a sustainable agriculture.

A World Commission on Environment and Development stated some time ago (York 1989): “Sustainable agriculture is to increase agricultural productivity and thus insure food security, while enhancing the productive capacity of this natural resource base in a sustainable manner”. Another similar statement made by York

(1989) considers: “Sustainable as the successful management of resources for agriculture to satisfy changing human needs, without degrading the environment or the natural resource base on which agriculture depends”.

The following statement by York (1989) is pertinent as a final comment:

“But the challenge of achieving sustainable agricultural systems around the world cannot be solved by agricultural interests alone. Indeed, sustainability is threatened far more by forces outside agriculture than from within”.

References

ABREU, A. F. de B.; M. A. P. RAMALHO, J. B. SANTOS & L. A. MARTINS. 1994. Progresso do melhoramento genético do feijoeiro nas décadas de setenta e oitenta nas regiões sul e alto Paranaiba em Minas Gerais. Pesquisa Agropecuária Brasileira, Brasilia, 29:105 – 112.

ALVIM, P. de T., 1999. Agricultura na Amazônia. (in press).

ARAUJO, J. S. de. 1995. Ganhos genéticos obtidos em híbridos e variedades de milho representativos de três décadas de melhoramento no Brasil. Diss. Mestrado, UFLA, Lavras.

AVERY, D. T. 1994. Saving the planet with high – yield farming. Proc. 49th Ann. Corn and Sorghum Industry Res. Conf.: 1 – 12.

BORLAUG, N. E. 1972. Human Population, Food Demands and Wildlife Needs. 37th North American Wildlife and Natural Resources Conf., Mexico City, March 12 – 15, 1972.

BREWBAKER, J. L. 1985. The tropical environment for maize cultivation. In: Breeding for Maize Production Improvement in the Tropics. Eds. A. Brandolini and F. Salamini, FAO and Istituto Agronomico per l´Oltremare, Firenze, Italia:47- 77.

CARVALHO, A. & L. C. FAZUOLI. 1993. Café. *In*: O Melhoramento de Plantas no Instituto Agronomico de Campinas, eds. A. M. C. Furlani & G. P. Viegas: 29 – 76.

EAST, E. M. 1908. Inbreeding in corn. Rept. Connecticut Agr. Exp. Sta. 1907: 419 – 428.

EMBRAPA. 1996. Centro de Pesquisa Agropecuaria dos Cerrados.

FAO, 1991. Production Yearbook. Table 1. Rome.

FAO, 1969. Production Yearbook Table 1. Rome.

FERNANDES, J. S. C. & J. F. FRANZON. 1997. Thirty years of genetic progress in maize (*Zea mays* L.) in a tropical environment, Maydica 42:21 – 27.

FERREIRA, M. & P. E. T. dos SANTOS. 1997. Melhoramento genético florestal dos *Eucalyptus* no Brasil: Breve histórico e perspectivas. *In*: IUFRO Conference on Silviculture and Improvement of *Eucalyptus*. Salvador, Anais, Colombo: EMBRAPA/CNPF 1:14 – 34.

FISHER, R. A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. Trans. Roy. Soc. Edinb. 52 : 399 – 433.

FISHER, R. A. 1936. Statistical methods for research workers. Sixth Edition Oliver and Boyd. Londres.

FLOR, H. H. 1955. Host – parasite interaction in flax rust – its genetics and another implications. Phytopath. 45:680 – 685.

KRATTIGER, A. 1998. The importance of Ag – biotech to global prosperity. ISAAA Briefs, Saskatoon, Canada: 1 – 10.

MACEDO J. 1995. Perspectives for the rational use of the Brazilian cerrado for food production. EMBRAPA/CPAC.

MACEDO, N., J. R. ARAÚJO & P. S. M. BOTELHO. 1993. Sixteen years of biological control of *Diatraea saccharalis* (Fabr.) (Lepidoptera: Pyrolidae) by *Cotesia flavipes* (Cam.)

(Hymenoptera: Braconidae), in the State of São Paulo. An. Soc. Entomol., Brasil. 22:441 – 448.

MALAVOLTA, E. 1999. Produção e produtividade – o impacto da pesquisa em fertilidade do solo, nutrição de plantas e adubação. (in press).

NEDEL, J. L. 1994. Progresso genético no rendimento de grãos de cultivares de trigo lançados para cultivos entre 1940 e 1992. Pesquisa Agropecuária Brasileira, Brasilia 29:1565-1570.

NILSSON – EHLE, H. 1909. Kreuzungsunter suchungen an Hafer und Weizen. Lunds Univ. Aarskr. N. F. Afd., Ser. 2, Vol 5, N.º 2:1-122.

PATERNIANI, E. 1990. Maize Breeding in the Tropics. In: Critical Reviews in Plant Sciences, 9:125 – 154.

RANGEL, P. H. N.; E. P. GUIMARÃES & P. C. F. N. NEVES. 1996. Base genética das cultivares de arroz (*Oryza sativa* L.) irrigado no Brasil. Pesquisa Agropecuária Brasileira, Brasilia 31:349-357.

SANCHEZ, P. A.; D. E. BANDY, J. H. VILLACHICA & J. J. NICHOLAIDES III. 1982. Amazon Basin soils: Management for continuous crop production. Science 216:821 – 827.

SHULL, G. H. 1909. A pure line method of corn breeding. Am. Breed. Assoc. Rept. 5:51-59.

SOARES, A. A.& M. A. P. RAMALHO. 1993. Estimativas do progresso genético no melhoramento do arroz (*Oryza sativa* L.). Comparação de métodos. Ciência e Práticas, Lavras 17: 27 – 34.

STORK, J. & W. D. TEAGUE. 1952. Flour for man´s bread. 382 p., University of Minnesota Press.

VENCOVSKY, R.; A. R. MORAES, J. C. GARCIA & N. M. TEIXEIRA. 1986. Progresso genético em 20 anos de melhoramento do milho no Brasil. *In*: Congresso Nacional de Milho e Soja, Belo Horizonte, Resumos: 300 – 307.

YORK, E. T. 1989. A Sustainable Global Agriculture. 1989. D. W. Brooks Lecture. The Univ. Georgia, USA, 15 p.

TABLE 1: COMPARATIVE CAPABILITIES OF VARIOUS TYPES OF AGRICULTURE IN RELATION TO HUNTING. (ADAPTED FROM STORK AND TEAGUE 1952 AND BORLAUG 1972).

SYSTEM	AREA REQUIRED (HA)	NUMBER OF PEOPLE FED
Hunting (1)	2500	1
Foraging (2)	250	1
Hoe Agriculture (3)	250	3
Plow Agriculture (4)	250	750
Modern Agriculture (5)	250	3600

(1) Indians of the North American plains (before European influence)

(2) California Indians (before European influence)

(3) Eastern wood-land Indians of North America (before European influence)

(4) Ancient Egyptian agriculture

(5) Highly developed modern agriculture of the USA (based on 1970 yields).

Table 2: Comparison between temperate and tropical climate for maize production.
(Adapted from Brewbaker 1985 and Paterniani 1990).

Characteristics	Temperate	Tropical
	GROWING CONDITIONS	
Yearly climatic variation	Relatively stable	Variable, unpredictable
Yearly rainfall variation	Relatively uniform	Variable, unpredictable
Rainfall among locations	Relatively uniform	Variable, unpredictable
Photoperiod	Longer days	Shorter days
Night temperature	Cooler	Warmer
Soil conditions	Usually favorable	Frequently adverse
Sowing period	Very restricted (few days)	Very broad (Several months)
Growing period	Well defined	Variable, broad
Germination constraints	Cold soil and fungi	Soil insects
Weed infestation	Medium	High
Insects of stored grain	Low infestation	High infestation, frequently started in the field
	TYPES OF MAIZE PLANTS	
Maturity cycle	Uniform adapted to the growing season	Variable, to adapt to climatic and socioeconomic situations
Plant height	Medium to short	Usually large
	INFRASTRUCTURE	
Distances among locations	Shorter	Larger
Transportation and communication	Usually satisfactory	Usually unsatisfactory
Number of researchers	Adequate	Inadequate

Table 3: Comparison among original varieties of coffee Arabica with improved and selected materials in São Paulo, Brazil. (Adapted from Carvalho and Fazuoli 1993).

Cultivar	yield	
	kg / há	%
Arabica	745	100
Red Bourbon	1333	179
Yellow Bourbon	1745	234
Mundo Novo (no selection)	1360	182
Mundo Novo (with selection)	2340	314

Table 4: Average gain in grain yield of maize due to genetic improvement according several evaluations.

Period	Yield increase kg/ha/year	References
1946 to 1986	60[1]	Paterniani 1990
1964 to 1983	72 to 109[2]	Vencovsky et al. 1986
1970 to 1990	31 to 51[1]	Araújo 1995
1964 to 1993	123[2]	Fernandes and Franzon 1997

[1] *Weight of grains*

[2] *Weight of ears*

Table 5: Grain and coffee production and Productivity in Brazilian Cerrados (EMBRAPA/CPAC 1996).

Crops	Production in 1000 ton (% Brazilian Production)		Increase	Productivity ton/ha		Increase
	1975	1993	%	1975	1993	%
Soybean	2.3 (3.1)	9.4 (41.5)	309	1.32	2.2	67
Maize	1.8 (17.3)	7.0 (23.2)	289	1.57	2.7	72
Rice	2.2 (42.8)	1.9 (19.1)	-.16	1.03	1.2	16
Bean	0.3 (13.1)	0.5 (19.9)	63	0.48	0.7	48
Coffee	0.08 (3.2)	0.5 (21.2)	575	0.82	1.3	62

Table 6: Production of main crops and beef in Brazilian "Cerrados" (Macedo 1995)

Activity	Area (10^6 ha)	Productivity (t / há / year)	Production (10^6 t)
Crops (no irrigation)	10.0	2.0	20.0
Crops (irrigation)	0.3	3.0	0.9
Beef (pastures)	35.0	0.05	1.7
Total	45.3		22.0

Table 7: Potential utilization of Continental Amazonian soils for agriculture. (Sanchez *et al* 1982).

Potential Use	Million hectares	%
Soils with no limitation	32	6
Usable soils with fertilizers	280	58
Soils practically not usable (1)	176	36
Total	488	100

(1) Due to topography, drainage and other physical limitations

The Role of Biological Nitrogen Fixation to Bio-Energy Programmes in the Tropics

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Introduction

The "Proalcool" programme initiated by the military regime after the two oil crises in the Seventies prompted a search for other renewable resources and their potential uses. Four million cars, vans, and small trucks in Brazil use ethyl alcohol produced by the fermentation and distillation of sugar cane for fuel. This is even mixed (22 percent) with petrol without damaging engines. On the other side, global society is based on oil which has been accumulating as subsoil reserves for thousands of years and will be finished soon (estimates 2050). In tropical regions, there is much more sun energy and therefore, chances of replacing petrol by biofuels are better potential alternatives. Progress made in all areas of biomass energy production, has been much greater per unit expenditure than progress achieved in the pursuit of nuclear fusion (Rosillo-Calle et al. 1994).

Sugar cane has been grown in Brazil for many decades with low or zero applications of nitrogen fertilisers. There are many areas in the country where sugar cane has been grown for decades, even centuries, and neither cane yields, nor soil N reserves, appear to fall with time, despite this apparent deficit in N supply. These results have led to research concerning the contribution of biological nitrogen fixation (BNF) to the maintenance of cane productivity. Recent results have shown that contributions up to 150 kg N ha^{-1} yr^{-1} can be obtained from the biological reduction of atmospheric nitrogen. Several nitrogen fixing bacteria colonise the hole plant and some of them live inside the plant which can fix the nitrogen and transfer it direct to the plant tissue. The screening of plant genotype for higher contributions of BNF has been cited to be the key to the replacement of N fertilisers in several important crops like sugar cane, rice, wheat, maize and others. Also diesel can be mixed with oil palm (around 20%) without any engines modification. African oil palm is the best alternative for the replacement and also is colonised by several nitrogen fixing bacteria. Biofuel are much more compatible with environmental preservation and as a renewable resource must be stimulated by the government.

The Brazilian Bio-Ethanol Programme

The elimination of N fertilisers for biofuel crops represents the key to high energy balances because these fertilisers are produced by the reduction of atmospheric N_2 to NH_4, using petrol or gas. The Brazilian ethanol programme is the best example of biofuel (Dobereiner, 1994). Sugar cane grown in Brazil for centuries, never received high N applications and therefore the genotypes grown today obtain significant contributions from biological N_2 fixation (BNF). When grown with ample P and K fertiliser and foliar application of molybdenum (500 g ha^{-1}) this crop may obtain more than 50 g N/m^2 during three years from BNF which means by extrapolating from the plot size of 2.7 m^2, the mean annual contribution to some commercial hybrids of sugar cane CB 45-3 and SP70-1143 a range from 170 to 210 kg N ha^{-1} (Table 1). These data confirm the differences between plant genotypes.

Sugar cane is now planted on 5.0 million ha in Brazil, 9% of the land under agriculture. With mean yields of 64 tons per ha, in addition to sugar, 10-12 billion litres of ethanol are produced per year, equivalent to 200.000 barrels of petrol per day (present situation). Although petrol prices all over the world currently are relatively low, the government of Brazil is convinced of the social and ecological impacts of the biofuel programme and plans to support it further. The key to the success of the Brazilian bio-ethanol programme is the high energy balance obtained in Brazil as shown in Table 2.

Table 1: Contributions of Biological N_2 fixation (BNF) to different sugar cane genotypes, evaluated by N Balance and ^{15}N dilution methods during tree years (Urquiaga et al.1992).

Cane Genotype	Total N accumulation in kg N/ha (3 yr)	BNF contribution in kg N/ha (3 yr)	
		N Balance	^{15}N dilution
CB 47-89	614 bc	397	348 c
CB 45-3	843 ab	626	526 b
NA 56-79	578 c	361	326 c
IAC 52-150	596 bc	379	338 c
SP70-1143	775 bc	558	519 b
SP71-799	569 c	352	333 c
SP70-2312	636 c	419	354 c
Chunnee	330 d	113	169 d
Caiana	11.6 d	- 101	67 d
Krakatau	1028 a	811	718 a

Nota: Differences between means significant at P = 0.001. NS, no significant differences between means at P = 0.05.

Due to the high N contributions the Brazilian sugar cane genotypes obtain from BNF, it is now recommended to the farmers to plant certain plant genotypes, CB 45-3 or SP70-11-43 without any N fertiliser and to use the money otherwise used for N fertilisers for increased phosphate applications, foliar spraying of Molybdenum, the key minor element for BNF, and irrigation. Elimination of leaf burning before harvest also increases the sugar cane yields and reduces the N applications as the leaves can contribute to the maintenance of the N in the system (Oliveira et al. 1994; Boddey, 1995). In addition it increases soil fertility and reduces irrigation needs. The higher labour need for cutting unburned sugar cane provides more jobs in the interior and costs are compensated by further increased yields.

The Brazilian Alcohol programme has already created more than one million jobs, decreasing the over-populations in large cities. Elimination of cane burning also will further reduce air pollution in addition to the negative greenhouse effect, by removing more CO_2 from the atmosphere. The use of biofuels has already reduced the lead content in the atmosphere of large cities by 75% and vehicles running on ethanol have zero lead emissions. Cars running on ethanol also emit 57% less CO, 64% less hydrocarbons and 13% less NOx than cars running on gasoline (Bohn, 1986). The only pollution problem is the smoke and soot produced by burning off the sugar cane trash (senescent leaves) before harvesting. Nowadays, more and more producers are using machinery's to harvest the sugar cane and around the cities it is already forbidden to burn, reducing further the pollution problems.

Ethanol production increased to 11,900 M litres by 1985 and by 1988, 88 per cent of the new cars being sold were powered by ethanol engines. It reduced drastically in 1995 to 3%. Unfortunately, with the decline in price of petrol, there were no interest of the government to continue this program (Figure 1).

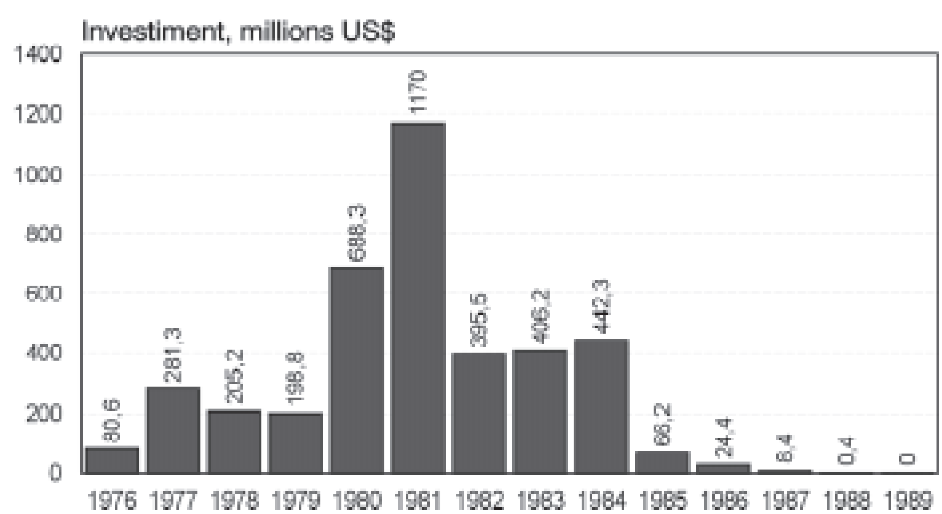
TABLE 2: ENERGY BALANCE OF ETHANOL PRODUCTION FROM SUGAR CANE UNDER BRAZILIAN CONDITIONS (BODDEY, 1995).

	ha^{-1} $year^{-1}$
a. Mean crop yield	65 t
b. Mean ethanol yields	3,564 litres
Energy produced	
c. Ethanol	18,747 Mcal
d. Residue (bagasse)	17,500 Mcal
e. Total (c + d)	36,297 Mcal
Energy expended	
f. Agriculture	4,138 Mcal
g. Factory	10,814 Mcal
h. Total (f + g)	14,952 Mcal
i. Energy gain (h - e)	21,345 Mcal
j. Overall energy balance ratio (e/h)	2.43
k. Net energy balance ratio assuming all factory power derived from bagasse[a] (c/f)	4.53
l. **Net energy balance ratio (k) but assuming** zero nitrogen fertiliser use[b]	5.79

[a] *Virtually all distilleries derive all heat and electricity from bagasse. Some sell excess bagasse as fuel to other neighbouring industries.*

[b] *Present mean N fertiliser use 65 kg^{-1}N ha^{-1} $year^{-1}$. Estimated energy cost 902 Mcal*

FIGURE 1: TOTAL BRAZILIAN GOVERNMENT INVESTMENT IN THE NATIONAL ALCOHOL PROGRAMME (PRO-ÁLCOOL) 1976 TO 1989 (BODDEY1993).



The key to the success of the Brazilian alcohol programme was the continuous selection of plant genotypes with N fertiliser applications much below the plant needs. With this approach, sugar cane genotypes were selected which associate with N_2 fixing bacteria using sun energy products of the plant to reduce the atmospheric N_2 into NH_4. N fertilisers are produced from atmospheric N_2 using petrol for the reduction process. High N applications, as recommended by the "Green Revolution" make any bio-energy programme senseless because the same amount of energy is used to make the biofuel as is obtained. For this reason, so far, Brazil is the only country in the world where biofuel programmes are energetically viable. The overall energy balance of ethanol production on Brazil is 2.5. If bagasse is used to produce all factory power, the energy balance increases to 4.5 and if in addition all N fertilisers are eliminated, it increases to 5.8 (Boddey 1993) With all these practices Brazil has been using the lowest N applications among all other countries (Dobereiner, 1997).

Palm Oil as an Alternative for Diesel Oil

The African oil palm (*Elaeis guineensis*) was introduced in Brazil, South of Bahia State in the XVI Century by the African slaves. Only in the 70´s, a stimulus in the production of this oil occurred due to the increase of its price in the world market and Pará State turned to be the highest producer in Brazil. Malaya is the biggest producer in the world contributing with more than 50% of the world production and also is the highest exporter country (64%). Brazil is responsible for only for 0.6% of the world production although palm oil the second one in production (18.49%) and consumption (20.40%) in the world.

The African oil palm seem to be the most interesting crop from the point of view of yield production. It may produces 4.0 - 8.4 t oil $ha^{-1}yr^{-1}$ and it has the highest energy yields (Table 3).

Another promised crop is peachpalm (*Bactris gasipaes*) that is also grown in the wet tropics and is already exploited commercially for palm hearts and its high yields of edible fruits. Besides oil, the starch meal can be converted into alcohol. This crop can also produces all over the year as the African oil palm with yield of 4.8 t oil $ha^{-1}yr^{-1}$ corresponding to 57 kcal $ha^{-1}.yr.^{-1}$ (Purseglove, 1968). In contrast, soybean produces only 0.6 t oil $ha^{-1}yr^{-1}$ during its crop cycle (120 days). Palm trees like African oil palm, can maintain a permanent large leaf area and root system and continuously produce, if well managed, for more then 20 years. This crop can also be harvested continuously throughout the year, requires a simple pressing process to produce the final fuel and release much less effluent to dispose of. It can also be grown on extremely poor soils and it is well adapted to wet tropic climate and represents a desirable tree alternative in forest areas. The main disadvantage is the high costs to establish the crop (1,866.21 US$/ha until the first harvest – Agrianual 97) and the long period of time (4 years) it takes until the first harvest.

Attempts have been made to convince the Brazilian government about the advantages of replacement of diesel oil by palm oil, which could create thousands of jobs for the poor population in the North and Northeast of the country. If the African oil palm would be planted in 30% of the area already deforested in the Amazon region, it could produce enough palm oil to replace all diesel oil used in Brazil which means around 460.000 barrels of diesel per day (Boddey, 1993).

TABLE 3: THE AGRICULTURAL AND ENERGY YIELDS OF THE MOST PROMISING CROPS (DOBEREINER ET AL., 1981)

	Crop yields t/ha.yr			Fuel yields [a]		Energy yields
	Actual means	Estimated potential	Type	L/t	L/ha.yr	TOE/ha.yr [a c]
Sugar crops						
Sugar cane	60	90	ethanol	67	4020	2.1
Sugar beet	30	56	ethanol	67	2010	1.0
Sweet sorghum (stems)	35	50	ethanol	55	1925	1.0
Starch crops						
Sweet sorghum (grains)	3	5	ethanol	30	1080	0.6
Cassava	13	40	ethanol	180	2340	1.2
Sweet potatoes	15	30	ethanol	125	1875	1.0
Maize	3	6	ethanol	360	1080	0.6
Babassu palm nuts	3[b]	25[b]	ethanol	80	240	0.1
Oil crops						
Babassu kernels	0.6	5	oil	37	22	0.02
African oil palm	18	38	oil	210	3780	3.7
Wood crops (25% H_2O)						
Eucalyptus or others	16	30	ethanol	159	2544	1.3
Eucalyptus or others	16	30	methanol	330	5280	2.7
Fire wood	16	30	wood	-	-	4.8

[a] *Calculated from present mean yields*

[b] *Present means are from extraction of native trees and potential are from cultivated plants.*

[c] *Calculated on the basis of: ethanol 5,120, methanol 5,034, oil 9,723 kcal/L and wood 3,000 kcal/kg and 10^7 kcal = 1 TOE.*

The possibility of running diesel motors on vegetable oil has been known since the work of Rudolf Diesel in 1911. The replacement of diesel oil by palm oil, which is used mainly in trucks and tractors would be of a tremendous beneficial effect to the environment, because oil palms take out more CO_2 from the atmosphere than would be returned by trucks, causing a negative greenhouse effect. Diesel oil can be mixed with up to 20 % palm oil without any need of change to the motor of trucks and buses. However, a further increase would require considerable modifications to the engine design, that are already available and have been sold by a German company Eslsbett Elsbett. Vusof Basiron and Ahmad Hitan estimated the cost of the use of oil palm in a car Mercedes 190 D using an Eslsbett Elsbett motor. A car powered with this engine runs 35,000 km without any technical problem using 6 litres of fuel per 100 km in urban area and 7 km on the road, with 30% superior performance than the normal diesel. The cost were estimated as 4.80 cents per km for oil palm fuel versus 5.87 cents using diesel.

Like in sugar cane, no high N fertiliser doses were applied on palm trees. The reason might be because commercial plantations of oil palms are mainly carried out in the poorest regions of the country that is, the North East and the Amazon region and because of the very little commercial use of these palms.

Species from the complex Orbignya-Attalea-Maximiliana are observed naturally in 16 million of hectares in Brazil and is normally called Babassu, which is actually the palm which also has a high extractive potential in the continent. Under natural conditions the productivity reaches around 150 litres of oil ha^{-1} and has a potential of up to 1 ton of oil per hectare after domestication. Babassu is exploited for the oil rich kernels and also the whole nut can be used as an energy source (IPT, 1979). There exists approximately 3,000 species of palm trees around the world of which about 1,600 occur in the American tropics region.

N_2 Fixing Bacteria Colonizing Sugar Cane and Oil Palms

Sugar Cane

During the 1950s two species of diazotrophic bacteria were found in high numbers in the rhizosphere of sugar cane. One of them was a new species called *Beijerinckia fluminensis* (Dobereiner and Ruschel 1958). These bacteria however only occur in soil and therefore the N_2 fixed by them is only partially available to the plant. In the 1970s a new genus, *Azospirillum* was described which also survives in soil and is enriched in the rhizosphere of various Gramineae including maize, rice, forage grasses, sugar cane and palm trees and which contains some specific strains which are able to infect the plant and multiply within plant tissues (Schloter et al., 1994).

Only at the end of the 80´s, aerial parts of plants, specially sugar cane that has a lot of carbon in the stems, was used for isolation and quantification of diazotrophic bacteria. This new habitat enabled the discovery of new species that colonise the plant interior without exhibiting any symptom of disease. In 1988, a new species of the Acetobacter family was found inside the sugar cane and was called *Acetobacter diazotrophicus* (Cavalcante and Dobereiner 1988, Gillis et al., 1989). Recently, these kind of organisms that live inside the plant tissue residing latently or actively colonising locally or systematically and do not showing visibly harm the plant and in some case improving plant growth and reduce disease symptoms caused by several plant pathogens, which do not survive in soil and which are transmitted within plant cuttings or seeds (Kado, 1992; Chen et al., 1995; Frommel et al., 1991; Kloepper et al., 1992; Pleban et al., 1995, Halmann et al., 1997). More recently however, two new species of N_2 fixing were reclassified as endophytes, (Dobereiner et al., 1994; Baldani et al., 1997) such as *Herbaspirillum seropedicae* (Baldani et al., 1986) and *H. rubrisubalbicans* (Gillis et al., 1990) and another new species was described colonising rice, maize and also sugar cane and was named *Burkholderia brasilensis* (Baldani, 1996).

A. diazotrophicus was first isolated from sugar cane and since then it was only isolated from *Pennisetum purpureum*, sweet potato and recently from coffee plants (Reis et al., 1994; Jimenez-Salgado, 1997). Comparing the survival outside the plant tissue from these endophytic bacteria, *A. diazotrophicus* was never found in the soil and inoculations of sterile and natural soil failed to isolate the bacteria (Baldani et al., 1997). It means that this organism needs the sugar cane tissue to survive and to pass to the next crop. In sugar cane, *Acetobacter diazotrophicus* was found colonising the roots, stems, leaves, trash (Reis et al., 1994) and internally was found in the xylem (James et al., 1994) and in the apoplast space in Cuba (Dong et al., 1994).

Using a model system to study the transference of the N fixed by this bacterium Cojho et al., (1993) used a mixed culture with a yeast and observed that more than half of the N_2 fixed by the bacteria could be liberated to yeast and suggesting that the plant also can obtain this amount of N. *Acetobacter diazotrophicus* seems well adapted to these sugar cane tissue as it shows the best growth with 10% sucrose and at pH 5.5. In addition, this bacteria does not poses a nitrate reductase, being able to fix N_2 in the presence of high levels of NO_3 (Cavalcante and Dobereiner, 1988). In the presence of 10% sucrose the NH_4 assimilation by this bacteria is only partially reduced (Boddey et al., 1991; Reis et al., 1998). This bacterium has also its nitrogenase activity only partially inhibited by ammonium (Stephan et al, 1991) and in the presence of 10% sucrose, the enzyme continues to fix nitrogen (Reis et al., 1998). Also, in the presence of high sucrose, the inhibition by oxygen, which damage the nitrogenase system, is less sensitive, maybe by the osmotic protection as the diffusion in the level of sucrose (10%) is reduced (Reis et al., 1998). These characteristics enable the bacteria to fix N_2 in complementation to N assimilation by the plant from soil.

In addition, two new species of *Herbaspirillum* were also found colonising endophytically sugar cane roots, stems and leaves (Baldani et al., 1996a). *Herbaspirillum seropedicae* was originally isolated from rhizosphere soil, washed roots and surface sterilised roots of maize, sorghum, and rice (Baldani et al., 1986), but not from uncropped soil (Baldani et al., 1992). *H. seropedicae* was originally thought to be a new species of *Azospirillum* by its similar growth characteristics in the semi-solid, N-free media. However, further analysis showed that it was a new genus (Baldani et al., 1986). Until now, this bacterium has been reported in 13 members of the Gramineae, normally colonising roots (Olivares et al., 1996) but was also found in the aerial parts of rice and maize as well in stems of sugar cane, but not in leaves (Olivares et al., 1996). In 1990, Gillis et al. reclassified *Pseudomonas rubrisubalbicans*, which causes the mottled stripe disease in sensitive sugar cane varieties, as *H. rubrisubalbicans*. With this new reclassification, another group was identified as "species 3" but includes only non-diazotrophic bacteria and is mainly isolated from clinical material, such as wounds and feces, although a few strains have been isolated from sugar cane, sorghum and maize (Baldani et al., 1996a). This two species of diazotrophic bacteria have very similar physiological characteristics and they can differ only in the utilisation of meso-erythritol (as sole carbon source) by *H. rubrisubalbicans* and N-acetylglucosamine by *H. seropedicae* and these characteristics are used to separate them. Also the optimal growth temperature (30ºC, *H. rubrisubalbicans*; 34ºC *H. seropedicae*) and by the use of oligonucleotide probes (Baldani et al., 1996a). *H. rubrisubalbicans* is less common, and could be isolated mainly from sugar cane and in less frequency from sorghum, (Hale and Wikie, 1972 a,b) rice, palm trees (Baldani et al., 1997) and *Miscanthus* (Kirchhof, et al., 1997).

Microscopy studies were performed to compare mottled stripe-disease susceptible variety of sugar cane (cv. B-4362 – Barbados) and the resistant one (cv. SP70-1143 – Brazil) and the difference was great. In the susceptible plant, the xylem vessel was completely blocked, intercellular spaces and substomatal cavities by the growth of *H. rubrisubalbicans.* In the resistant variety SP70-1143, bacteria were restricted to microcolonies encapsulated within polymeric material (Olivares et al., 1997). James et al., (1997) inoculated this two species of bacteria in sorghum leaves and observed that both posses the same behaviour, forming microcolonies in sorghum leaves. A complete review of this two endophytic bacteria was done by James and Olivares, (1997).

Recently, another new endophytic N_2-fixing bacterium, *Burkholderia brasilensis* which grows best at pH 4.5-5.5 was described by Baldani et al. (1996b). This species was also isolated from sweet potato, rice and cassava. Also, a few strains of another species of *Burkholderia* were isolated from sugar cane plants collected from Pernambuco. These strains are very close related to *B. brasilensis* and any the physiological testes could differentiate them until now. The only way is to use the probes produced for each type strain using 23S rRNA variable sequence. This result suggest that these strains belong to two different species (Kirchhof et al., 1997).

The endophytic occurrence of these diazotrophs may now explain the high contributions sugar cane can obtain from BNF observed by Lima et al., (1987), Urquiaga et al., (1992) and Yoneama et al., (1997). The amount of N obtained from BNF and the difference between cane genotypes is shown in Table 1.

Palm trees

Until now, only two reports showed the presence of diazotrophic bacteria belonging to the *Azospirillum amazonense* species were found colonising roots of palm trees (Magalhães et al., 1983; Magalhães and Dobereiner, 1984). Recent, attempts were carried out to isolate similar endophytic diazotrophs from palm trees at various sites in Brazil including Amazon and South Bahia (Table 4). Numbers of diazotrophic bacteria were higher in roots as compared to the other part of the plants. Also the endosperm of the seed is colonised by these microorganisms. Oil palm (*Elaeis guineensis* - Dendê) and Peachpalm (*Bactris gasipaes* - Pupunha) are colonised by *Azospirillum brasilense*, *A. amazonense*, *A. lipoferum*, *Herbaspirillum seropedicae* occurred in oil palm while *Azospirillum brasilense*, *A. amazonense*, *A. lipoferum* and *Beijerinckia* spp. were found colonising peachpalm. Also other as-yet-unidentified N_2-fixing bacteria were present in these two palm trees. These unidentified bacteria are present in the roots, stems, leaves and in the endosperm of the fruit. Preliminary results suggest that a new *Herbaspirillum* species is present in roots, stems and leaves of these palm trees (Ferreira et al. 1995). In the literature, there is only one report showing occurrence of *Azospirillum* spp. in oil palms grown in Malaya (Shamsuddin et al., 1995).

Table 4: Occurrence of diazotrophic bacteria in tree different genotypes of oil palm collected in the South Region of Bahia State (Numbers of cells per gram fresh weight) (Carvalho and Dobereiner, in preparation).

Genotype	Washed Roots		Sterilized Roots		Leaves	
	JNFB	LGI	JNFB	LGI	JNFB	LGI
Dura	1.3×10^5	2.0×10^5	6.2×10^3	n. d.	n. d.	1.0×10^2
Tenera	4.1×10^6	1.5×10^5	1.4×10^5	1.9×10^4	1.0×10^2	1.0×10^2
Native	5.3×10^6	5.6×10^4	n. d.	n. d.	9.0×10^2	n. d.

JNFB semi-specific media used for isolation of Herbaspirillum *species.*

LGI semi-specific media used for isolation of A. amazonense.

Carvalho, (1997) used a mixture of strains including *H. seropedicae* (Z67), *Burkholderia brasilensis* (M130) and *A. lipoferum* (Sp260) in an inoculation experiment with African oil palm and peachpalm plants. The author also used a mixture of 3 isolates from African oil palm including 2 strains of *Herbaspirillum* (8A and 7C) and one strain of *A. brasilense* (23B). These experiments also included a treatment using mycorrhizae fungi *Glomus clarum* alone or in a mixture with the diazotrophic bacteria. These plants were dependent on N fertilisation during the first 6 months and the AM fungi increased N assimilation by the peachpalm and the African oil palm. The inoculation with diazotrophic bacteria alone or with AM fungi showed a better effect than the uninoculated control without nitrogen but it was lower than the N fertilisation for all parameters analysed.

The author also applied the same treatments to oil palm plants replacing one treatment by a mixture of 3 species of arbuscular mycorrhizal fungi and a mixture of strains: *A. lipoferum* Sp260 and Br 17; *H. seropedicae* Z67 and *B. brasilensis* M130 and the isolates from palm trees. An increase in stem height, stem diameter, height of the first leaf, leaf area and weight of the dry shoot and total N in roots was observed with inoculation of a mixture of all bacterial strains. It suggest that a better combination of diazotrophic bacteria must be tested.

Perspectives for the Future

All these new findings open perspectives for a replacement of diesel oil as well as gasoline by bio-energy sources which are much more compatible with environmental preservation and are renewable resources. The substitution of these derivatives of petroleum is necessary to overcome these problems and the solution is renewable energy sources which are clean and originate from biomass. This is only possible in countries which possess sufficient reserves of land for the expansion of crop production along with a suitably warm climate and an abundance of rainfall as observed in Brazil and Nigeria

In the Conference held in Brazil in 1992 (ECO-92), the developed countries made several promises to reduce the global effects of the use of fossil fuels but this has not been done. Of course is not easy to change the society as the cost of petrol does not provide the necessary motivation. Also, the countries which are localised in temperate region have their lands are fully occupied. The Brazilian government must stimulate the use of these natural energy sources and also sell this technology or the product (ethanol) for the others, especially to cities where the air pollution is too high, such as Mexico city. Of course, the government must invest money to maintain the progress and reduce the global alterations in America. The use of oil palm oil as a fuel is the best option to reduce the use of imported oil as it is the requirement for diesel oil that is mainly responsible for the high demand for imported crude oil. Palm oil would be an ecological solution to replace diesel oil imported to the Amazon region.

In any case, a energy balance must be positive otherwise it is not viable. Biological nitrogen fixation can reduce the use of N fertiliser which is the most expensive and also needs fossil energy for its production. In sugar cane, even not replacing nitrogen fertiliser by BNF, the cost of production in Brazil are already the lowest in the world,. Here, the N fertilisation adopted by the farmers averages 60 kg N/ha and the productivity in the State of São Paulo (where 60 % of Brazilian sugar cane is grown) is around 80 ton fresh stems per ha. In United States, Australia, Mexico, India (maybe others) the N fertilisation is around 200-300 kg/ha. In Australia, using 200 kg N/ha and irrigation in much of the area, the productivity is only slightly above that of São Paulo (84 ton/ha). In these countries, the energy balance is much less favourable, even negative in some cases, and ethanol production from this crop is not viable (Pimentel et al., 1988).

In Brazil alcohol has been used as a fuel to replace gasoline in cars and light vehicles for almost 20 years and the air pollution of São Paulo city was considerably reduced (Bohm, 1986). Another important reason that we can not forget is that the oil from the African oil palm can be produced in the northern region were the cost of transportation of fuels is high. The PROALCOOL program together with the "DENDIESEL" program need political support.

References

Agrianual 97. Anuário da Agricultura Brasileira. FNP Consultoria e Comércio.

Baldani, J. I., Baldani, V. L. D., Seldin, L., and Dobereiner, J. 1986. Characterization of *Herbaspirillum seropedicae* gen. nov. sp. nov. a root associated nitrogen fixing bacterium. *International Journal of Systematic Bacteriology*, **36**: 86-93.

Baldani, J. I., Pot, B., Kirchhof, G., Falsen, E., Baldani, V. L. D., Olivares, F. L., Hoste, B., Kersters, K., Hartmann, A. Gillis, M., and Dobereiner, J. 1996. Emended description of *Herbaspirillum*; inclusion of (*Pseudomonas*) *rubrisubalbicans*, a mild plant pathogen, as *Herbaspirillum rubrisubalbicans* comb. nov. and classification of a group of clinical isolates (EF group 1) as *Herbaspirillum* species 3. *International Journal of Systematic Bacteriology*, **46:** 802-810.

Baldani, J.I., Caruso, L., Baldani, V. L. D., Goi, S. R., and Dobereiner, J. 1997. Recent advances in BNF with non-legume plants. *Soil Biology and Biochemistry*, **29:** 911-922.

Baldani, V. L. D. 1996. Efeito da inoculação de *Herbaspirillum* spp. no processo de colonização e infecção de plantas de arroz e, caracterização parcial de uma nova bactéria diazotrófica e endofítica do gênero *Burkholderia*. PhD. Thesis. Universidade Federal Rural do Rio de Janeiro.

Baldani, V. L. D., Oliveira, E., Balota, E., Baldani, J. I., Kirchhof, G. and Dobereiner, J. 1996. *Burkholderia brasilensis* sp.nov. uma nova bacteria diazotrofica endofitica. Anais da Academia Brasileira de Ciências (in press).

Baldani, V. L. D., Baldani, J. I., Olivares, F. L., and Dobereiner, J 1992. Identification and ecology of *Herbaspirillum seropedicae* and closely related *Pseudomonas rubrisubalbicans*. *Symbiosis*, **13**: 65-73.

Boddey, R. M.(1993). Green energy from sugar cane. *Chemistry and Industry* **17**: 355-358

Boddey, R. M., Urquiaga, S. Reis, V. M. and Dobereiner, J. 1991. Biological nitrogen fixation associated with sugar cane. *Plant and Soil* **37**: 111-117.

Bohm, G. M. Impacto da poluição de veículos automotores na saúde e meio ambiente. FIESP/CIESP, São Paulo, 1986.

Carvalho, A. R. V. 1997. Associação de bactérias diazotróficas e fungos micorrízicos arbusculares em mudas de pupunheira (*Bactris gasipaes* H. B. K.) e de dendezeiro (*Elaeis guineensis* Jaquim.) Ms Thesis. Universidade Federal Rural do Rio de Janeiro. 264 p.

Cavalcante, V. A. and Dobereiner, J. 1988. A new acid tolerant bacterium associated with sugar cane. *Plant and Soil* **108:**23-31

Chen, C., Bausdke, E. M., Musson, G., Rodrígues-Kábana, R., and Kloepper, J. W. 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. *Biological Control* **5**: 83-91.

Cojho, E. H., Reis, V. M., Schenberg, A. C. G., and Dobereiner, J. 1993. Interactions of *Acetobacter diazotrophicus* with an amylolytic yeast in nitrogen free batch culture. *FEMS Microbiology Letters* **106:** 341-346.

Dobereiner, J. and Ruschel, A. P. 1958. Uma nova espécie de *Beijerinkia. Revista de Microbiologia* **1:** 261-272.

Dobereiner, J. 1994. Alternatives for biofuel production: The Brazilian example. Pontifitiae Academia Cienciarum.

Dobereiner, J., Araújo Neto, J. S., and Arkoll, D. B. 1981. Energy alternatives from agriculture. *Pontificiae Academiae Scientiavm Scripta Varia* **46:** 433-458.

Dobereiner, J., Baldani, V. L. D., Olivares, F. L., and Reis, V. M. 1994. Endophytic diazotrophs: The key to graminaceous plants. *In*: Nitrogen fixation woth non-legumes. Eds N. A. Hegazi, M. Fayez, and M. Monib. pp. 395-408. American University of Cairo Press. Cairo, Egypt.

Dong, Z., Canny, M. J., McCully, M. E., Roboredo, M. R., Cabadilha, C. F., Ortega, E., and Rodés, R. 1994. A nitrogen fixing endophyte of sugarcane stems. *Plant Physiology* **105:** 1139-1147.

Ferreira, A. C., Cozzolino, K., Carvalho, A. R. V., and Dobereiner, J. 1995. Isolation and characterisation of diazotrophic bacteria in oil palm trees. In *International Symposium on Sustainable Agriculture for the Tropics - The role of Biological Nitrogen Fixation* pp. 210-211. Boddey, R. M. and Resende, A. S., Eds. EMBRAPA, Rio de Janeiro.

Frommel, M. I., Nowak, J., and Lazarovits, G. 1991. Growth enhancement and development modifications of *in vitro* growth potato (*Solanum tuberosum* spp. *tuberosum*) as affected by a nonfluorescent *Pseudomonas* sp. *Plant Physiology* **96:** 928-936.

Gillis, M., Dobereiner, J., Pot, B., Goor, M., Falsen, E., Hoste, B., Reinhold, B., and Kersters, K. 1990. Taxonomic relationships between *Pseudomonas rubrisubalbicans*, some misnamed clinical isolates (EF group 1) and *Herbaspirillum seropedicae*. *In*: Nitrogen Fixation, pp. 293-294. Pollinelli, M., Materassi, R., and Vincenzini, M. Eds. Kluwer, Dordrecht.

Gillis, M., Kersters, K., Hoste, B. Janssens, D., Koppenstedt, R. M., Stephan M. P., Teixeira, K.R., Dobereiner, J. and De Ley, J.(1989). *Acetobacter diazotrophicus* sp.nov., a nitrogen fixing acetic acid bacterium associated with sugar cane. *International Journal of Systematic Bacteriology* **39:** 361-364.

Hale, C. N. and Wikie, J. P. 1972 a. A comparative study of *Pseudomonas* species pathogenic to sorghum. *Nova Zelandia Journal Agriculture* **15:** 448-456.

Hale, C. N. and Wikie, J. P. 1972 b. Bacterial leaf stripe of sorghum in New Zeland. *Nova Zelandia Journal Agriculture* **15:** 457-460.

Halmann, J., Quadt-Hallmann, A., Mahaffee, W. F., and Kloepper, J. W. 1997. Bacterial endophytes in agricultural crops. *Canadian Journal Microbiology* **43:** 895-914.

I. P. T. Análise tecnológica, econômica e social do aproveitamento integral de côco de Babaçu. Instituto Pesquisas Tecnológicas, São Paulo. v. 2 (1979).

James, E. K. and Olivares, F. L. 1998. Infection and colonisation of sugar cane and other graminaceous plants by endophytic diazotrophs. *Critical Review in Plant Science*, **17:** 77-119.

James, E. K., Olivares, F. L., Baldani, J. I., and Dobereiner, J. 1997. *Herbaspirillum*, an endophytic diazotroph colonising vascular tissue in leaves of *Sorghun bicolor* L. Moench. *Journal of Experimental Botany,* **48:** 785-797.

James, E. K., Reis, V. M., Olivares, F. L., Baldani, J. I., and Dobereiner, J. 1994. Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. *Journal of Experimental Botany,* **45:** 757-766.

Jimenez-Salgado, T., Fuentes-Ramirez, L. E., Tapia-Hernandez, A., Mascarua-Esparza, M. A., Martinez-Romero, E., and Caballero-Mellado, J. 1997. *Coffea arabica* L., a new host plant for *Acetobacter diazotrophicus* and isolation of other nitrogen-fixing Acetobacteria. *Applied and Environmental Microbiology,* **63:** 3676-3683.

Kado,C. I. 1992. Plant pathogenic bacteria. In: The prokaryotes. Balows, A., Truper, H. G., Dworkin, M., Harder, W., and Schleifer, K. –H. Eds. Spring-Verlag, New York. pp. 660-662.

Kirchhof, G., Reis, V. M., Baldani, J. I., Eckert, B., Dobereiner, J., and Hartmann, A. 1997. Occurrence, physiology and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. *Plant and Soil,* **194:** 45-55

Kloepper, J. W. and Beauchamp, C. J. 1992. A review of issues related to measuring colonisation of plant roots by bacteria. *Canadian Journal Microbiology*, **38**: 1219-1232.

Lima, E., Boddey, R. M., and Dobereiner, J. 1987. Quantification of biological nitrogen fixation associated with sugar cane using a ^{15}N aided nitrogen balance. *Soil Biology and Biochemistry*, **19**: 165-170.

Magalhães, F.M., Baldani, J.I., Souto, S.M., Kuykendall, J.R., Dobereiner, J. 1983. A new acid-tolerant *Azospirillum* species. *Anais da Academia Brasileira de Ciências*, Rio de Janeiro, 55: 417-430.

Magalhães, F. M. and Dobereiner, J. 1984. Ocorrência de *Azospirillum amazonense* em alguns ecossistemas da Amazônia. *Microbiologia* **15**: 246-252.

Olivares, F. L., Baldani, V. L. D., Reis, V. M., Baldani, J. I., and Dobereiner, J. 1996. Occurrence of the endophytic diazotroph *Herbaspirillum* spp. in roots, stems and leaves predominantly of Gramineae. *Biology and Fertility of Soils*, **21**: 197-200.

Olivares, F. L., James, E. K., Baldani, J. I., and Dobereiner, J. 1997. Infection of mottled stripe disease susceptible and resistant varieties of sugar cane by the endophytic diazotroph *Herbaspirillum*. *New Phytoogist,* **135**: 723-737.

Oliveira, H.P., Urquiaga, S. Boddey R.M. (1994) Burning sugar cane: The long term effects. Int. *Sugar Journal,* **96**: 272-275

Pleban , S., Ingel, F., and Chet, I. 1995et al., 1995. Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the greenhouse using endophytic *Bacillus* spp. *European Journal of Plant Pathology* **101**: 665-672.

Pimentel, D., Warneke, A. F., Teel, W. S., Schwab, K. A., Simcox, M. J., Ebert, D. M., Baenisch, K. D. and Aaron, M. R. 1988. Food versus biomass fuel: Socio-economic and environmental impacts in the United States, Brazil, India and Kenya. *Advances Food Research* **32**: 185-201.

Purseglove J.V. (1968). Tropical crops: Dicotyledons and Monocotyledons. Longmans (London).

Reis, V. M. and Dobereiner, J. 1998. Effect of high sugar concentration on nitrogenase activity of *Acetobacter diazotrophicus*. *Archives in Microbiology*, **171**: 13-18

Reis, V. M., Olivares, F. L., and Dobereiner, J. 1994. Improved methodology for isolation of *Acetobacter diazotrophicus* and confirmation of its endophytic habitat. *World Journal of Microbiology and Biotechnology,* **10**: 101-104.

Rosillo-Calle F., Hall D.O., Arora, A. L. Carioca, A.(1994). Bio-ethanol production: Economic and social considerations in failures and successes. pp 23-53. In: Biotecnology: Economic and Social Aspects. Issues of Developing Countries. Da Silva E. J., Radledge, C., Sasson, A., Cambridge Univ. Press.

Schloter, M., Kirchhof, G., Heinzmann, U., Dobereiner, J., and Hartmann, A. 1994. Immunological studies of the wheat root colonisation by *Azospirillum brasilense* strains Sp7 and Sp245 using strain-specific monoclonal antibodies. In: *Nitrogen-Fixation with Non-Legumes.* Hegazi, N.A., Fayez M., and Monib, M., Eds., pp. 291-297. The American University in Cairo Press, Cairo.

Shamsuddin, M. M., Rohani, O., Roslina, A. W., Ho, C. N., Noraza, H. Chalk, P. M. Nitrogen fixation by *Azospirillum* in association with oil palm (*Elaeis guineensis* Jaquim.). In *International Symposium on Sustainable Agriculture for the Tropics - The role of Biological Nitrogen Fixation* pp. 232-233. Boddey, R. M. and Resende, A. S., Eds. EMBRAPA, Rio de Janeiro.

Solos e Adubos. Boletim Informativo (1988). São Paulo 21, 147.

Stephan, M. P., Oliveira, M., Teixeira, K. R. S., Martinez-Drets, G. and Dobereiner, J. 1991. Physiology and dinitrogen fixation of *Acetobacter diazotrophicus. FEMS Microbiology Letters.* **77**: 67-72.

Urquiaga S., Cruz K. H. S., Boddey R.M. 1992. Contribution of nitrogen fixation to sugar cane: Nitrogen-15 and nitrogen balance estimates. *Soil Science Society of American Journal*, **56**:105-114

Yoneama, T., Muraoka, T., Kim, T. H., Davanay, E. V., and Nakanishi, Y. T. 1997. The natural 15N abundance of sugarcane and neighbouring plants in Brazil, the Phylippines and Miyako (Japan). *Plant and Soil* **189**: 239.244.