SCIENCE NEWS

NATIONAL SYMPOSIUM ON HARNESSING BIOLOGICAL NITROGEN FIXATION TO ENHANCE AGRICULTURAL PRODUCTIVITY

(HELD AT IARI, NEW DELHI, FEBRUARY 25-27, 1982)

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IN ORDER to assess the research and developmental work in India on biological nitrogen fixation (BNF) and to encourage interaction and coordination between the scientists involved, the Food and Agriculture Committee of the Department of Atomic Energy organised a National Symposium at the Indian Agricultural Research Institute, New Delhi, during February 25-27 1982. Nearly 200 scientists participated in the symposium which was divided into twelve sessions covering genetics, physiology, biochemistry, pesticide effects and field studies of free-living, associative and symbiotic nitrogen-fixing organisms. A theme session on the biotechnologies for nitrogen fixation and open forum sessions on the topics covered on each day were the novel aspects of the symposium. The salient features of the symposium were evaluated during a panel discussion and an assessment of the main points made is summarised here.

Aspects needing early decisions at the national level

The papers presented by G. S. Venkataraman and his associates (IARI) and by P. K. Singh (CRRI) stressed the benefits that the average and marginal rice farmer would gain by the application of biofertilizers. Proper extension machineries, preferably separate arrangements for cyanobacteria and *Azolla*, should be organized to popularize these techniques among farmers.

The work of N. S. Subba Rao (IARI), Peter Dart (ICRISAT, Patencheru, A.P.) and Patrick Tauro (Haryana Agricultural University, Hissar) and their associates highlighted the immense potential for *Rhizobium* inocula in India. Mass production of *Rhizobium* inocula has been a routine commercial activity in many advanced countries. The relative merits of the production technologies practised in USA and Australia and their suitability under Indian conditions need a thorough examination. A National Culture Collection Centre for nitrogen-fixing microbes, should be set up from where authentic cultures can be obtained by interested institutions or individuals.

Microbial inocula are now regularly supplied by public and private institutions but the quality suffers even though ISI standards have been specified in the case of *Rhizobium*. Rigorous quality control is 'a must' to ensure that sub-standard microbial inocula do not reach the farmer and destroy the credibility of biofertilizers. An independent laboratory with trained personnel should be set up to assess and certify microbial inocula. Insufficient sterilisation of carrier materials, for *Rhizobium* results in heavily contaminated inocula with inadequate *Rhizobium* count. J. A. Thompson (ICRISAT) drew attention the effective use of gamma irradiation in Australia for bulk sterilisation of carrier materials. Setting up national facility, for this purpose should be explored with the Bio-Medical Group, BARC, where suitable facilities and expertise are available. The immense potentialities of molecular and biochemical genetics in nitrogen fixation research were stressed in the keynote address by Dr. H. K. Jain and by all speakers in the theme session. The appropriate decisions for strengthening such research should be made now, so that we are not left behind in this area.

Short-term research goals for enhancing biological nitrogen fixation

Several constraints and unsolved problems hinder the application of biofertilizers. The experience of HAU and ICRISAT indicate that in semi-arid regions, water stress, temperature and humidity are some of the main factors which control effective nodulation. Reliable techniques, such as isolation of strain specific antigens in combination with enzyme-linked immunosorbent assay (ELISA) have to be developed and standardised to ascertain that the inoculated rhizobia as also cyanobacteria and other microbes have really established in the farmer's field. Competition from indigenous microbes thwart the effectiveness of the inocula. Specific answers to competition from indegenous microbes would emerge if field investigations and physiological and biochemical studies in this area are complemented by genetic analysis including those of the plant cultivars. Pulses are grown after paddy in nearly two million hectares per year and rhizobia are often eliminated during soil flooding resulting in a situation where legumes would readily respond to seed inoculation with *Rhizobium*. The effect of paddy cultivation on *Rhizobium* population and the benefit

from inoculation should be a priority area of research.

Increased crop yields have been obtained using microbial inocula even when the normal quantity of fertilizer N is added. Whether this is due to an incremental amount of atmospheric N_2 fixed should be ascertained for various cropping systems by collecting data using ^{15}N isotope. A major problem with *Azolla* is the inability of the dried fern to regenerate. It appears that sporocarp bearing *Azolla* can be composted and such material would regenerate effectively. This lead and other avenues should be explored to develop dry *Azolla* inocula. Selection and breeding for *Azolla* strains insensitive to light and temperature are attractive research goals.

Associative symbiosis mediated by *Azospirillum* and rhizosphere microorganisms may contribute significantly to nitrogen fixation in cereal and grass ecosystems. However, most of the data available are based on long-term acetylene reduction measurements which are subject to severe artifacts. Extensive data on the lines presented by V. R. Rao, (CRRI, Cuttack) using ^{15}N need to be obtained. The consensus of international scientific opinion has been that *Azotobacter* contributes only insignificantly by way of nitrogen fixation. However, it would be worthwhile to obtain more data under tropical field conditions as indicated by the work at IARI and of M. Lakshmanan's group at Madurai Kamaraj University. The role of nodule-bearing plants such as *Leucaena*, *Sesbania* and *Casuarina* are well known in reforestation, and in fodder and fuel wood production. The suitability of these and other promising nitrogen-fixing shrubs and trees in different ecogeographical zones of the country should be assessed.

Medium range research goals

In spite of the major advances in the physiology and biochemistry of nitrogen fixation many unknown facets still remain to be elucidated. For instance, S. K. Apte, J. Thomas and T. Fernandes (BARC) have found that in cyanobacteria, sodium is an essential requirement for nitrogenase action, probably involved in the activation of the enzyme. Further, research leading to a fuller understanding of the regulation of nitrogenase is a prerequisite for its optimum exploitation in agriculture.

In legumes the need for extending the period of nitrogen fixation during the entire plant growth phase has been apparent. Recent biochemical studies by M. S. Naik and associates (IARI) on groundnut mutants developed at BARC, have shown that there is a wide variability in the plant germ plasm for N_2 fixation potential. Remarkably, some cultures continue to fix nitrogen during the whole plant growth cycle. Selection and breeding of cultivars for optimizing nitrogen fixation in consonance with other beneficial plant and bacterial attributes are evidently attractive and useful research programmes. Experiments by K. Dadarwal (HAU, Hissar) indicate that cowpea group legumes nodulated by Hup^+*Rhizobium* strains contain less leghemoglobin (Lb), yet are more effective in N_2 fixation. It is suggested that the quantity of Lb in nodules is not a safe parameter of effectivity. The relative efficiency of the various pathways involved in nitrogen assimilation in tropical legumes, needs to be ascertained. Recent work with soyabean has highlighted the better carbon/nitrogen economy of such plants having ureide as the main N component, transported out of nodules. The area of early interactions in legume—*Rhizobium* symbiosis is a virtual black box. The promising lead obtained by A. Bhagwat and J. Thomas (BARC) with cowpea, indicate that legumes secrete signal compounds which seem to favour selectively the synthesis of specific polysaccharides (essential for infection and nodulation) in the rhizobial partner. This needs further investigation.

Long term research goals

In recent years, recombinant DNA analyses, transposon mutagenesis and related tools of genetic engineering, have revealed many facets of the gene complex (*nif*), involved in nitrogen fixation. Transfer and stable expression of *nif* genes in previously non-N_2-fixing prokaryotes have been achieved and promising leads are available for attempting *nif* transfer to eukaryotes. The specificity barrier in legume—*Rhizobium* symbiosis has been broken with the employment of new strains, each capable of nodulating more than one legume; and of other strains, all capable of nodulating a given legume. Nevertheless, we still know very little about the location, characteristics and functions of an estimated twenty *sym* (symbiotic) genes which determine host recognition and several other steps which precede effective nodulation and N_2 fixation.

The breakthroughs needed for enhancing N_2 fixation in existing systems, and in extending it to other microbes and crop plants, have to come through genetic manipulation. Although we are far behind in this area, some of the papers provided significant new information on the regulation of *nif* expression. G. Narsiah and H. K. Das (JNU, New Delhi) demonstrated the transcription and translation of *nif*HDKY, cloned in *E. coli*, independent of any *nif* specific positive control element, such as the *nif* AL operon. Recombinant DNA analyses of the *gln*A region of *E. coli* by R. Tuli and J. Thomas (BARC) have enabled the identification of *ntr* genes, the primary regulatory genes involved in the expression of *gln*, *hut* and *nif* (genes for glutamine synthetase, histidase and nitrogenase respectively) operons. More importantly, they found that the segment near the N-terminal end

of *gln*A was required in addition, for full derepression of *nif* and constructed a *Klebsiella pneumoniae* strain, constitutive for nitrogenase.

The questions amenable to molecular genetic research tools are many more. Can we engineer efficient N_2 fixing cereals, oxygen insensitive nitrogenase, more efficient systems than the *Azospirillum*—grass rhizocoenosus, leghemoglobinless but effective non-legume—*Rhizobium* symbiosis as in *Parasponia*, or stem nodules, where the *Rhizobium* inoculant does not have to cope with competition from soil bacteria? Answers may have to await decades of research, but we need to take the plunge and organize strong research schools because, more and more of the brilliant scientists involved in such work in the West are entering commercial enterprises from where, the free flow of information, which we now take advantage of, may soon dry up.

ANNOUNCEMENTS

THIRD EUROPEAN COMMUNITY SYMPOSIUM IN INNOVATION FINANCING

The Symposium will be held at Luxembourg during 17–19 November, 1982. The introduction of new technologies in the market presupposes investments which it is difficult for newly-established enterprises to finance. On the other hand, new technologies hold the key to improving the competitiveness of national economies and to guaranteeing employment on a long-term basis. For this reason, solutions to this financing problem are being sought throughout the European Community.

The Third annual European Community Symposium on innovation financing, which will be held in Luxembourg during 17–19 November, 1982, wil cover the theme 'Needs of new technology-based enterprises'. It will bring together enterpreneurs and financiers who will discuss recently devised financing models on the basis of acquired experience. The programme will cover: risk capital and the financing of buy-outs, national public guarantee schemes, examples for mobilising private interest and local support, and the tailoring of financial packages tb needs.

Further information may be had from J. M. Gibb, C.E.C. DG XIII, P. B. 1907, Luxembourg.

TWELFTH SYMPOSIUM ON FUSION TECHNOLOGY

The Symposium on Fusion Technology will be held at Julich (FRG) during 13–17 September, 1982. Further particulars may be had from the Conference Secretary, Ms. H. BONGARTZ, KFA Julich GmbH Tagungsburo, Postfach 1913, D-5170–Julich 1. F R G.

SECOND CONFERENCE IN BIOMASS

The Conference will be held during 20–23 September, 1982 at Berlin. Further particulars may be had from Dr. A. Strub, Commission of the European Communities DG XII, 200 rue de la Loi, b–1049 Bruxelles, Belgium.

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