CURRENT STATUS OF BIOLOGICAL NITROGEN FIXATION RESEARCH

(NATIONAL SYMPOSIUM)

6-8 Feb 1986

1986

Edited by :

RANDHIR SINGH, H. S. NAINAWATEE & S. K. SAWHNEY

Department of Chemistry & Biochemistry
Haryana Agricultural University
Hisar-125 004

Food and Agriculture Committee
Department of Atomic Energy
Government of India

and

Haryana Agricultural University
Hisar

CP 236

MEASUREMENT OF NITROGEN FIXATION

S. P. Wani
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, P.O., Andhra Pradesh-502 324, India.

Measurement of biological nitrogen fixation (BNF) is essential to select host cultivars and microbial strains efficient in nitrogen fixation and to study the effects of biological and environmental factors on BNF. Methods for measurement of BNF include: growth in N-free or N-deficient systems, morphological determinations, N analysis (including isotopic methods), metabolic product determinations, and reduction of alternate nitrogenase substrates. As each method has certain limitations, advantages, the choice of a particular method depends on the objectives of the study.

In earlier studies, increased growth as biomass or optical density in N-free media were used as the simplest criteria in classifying nitrogen fixers. It is now generally agreed that this approach is not a satisfactory criterion for nitrogenase activity as it often results in erroneous classification (1). Some organisms, with a low N requirement but efficient in scavenging traces of combined N in a medium, can grow in a relatively N-deficient medium (2). Conversely, some N_2-fixing bacteria cannot grow on a medium completely devoid of combined N (3). The presence of specialized cells called heterocysts has been correlated with N_2 fixation by blue green algae; however, N_2 fixation occurs in unicellular algae, and in a filamentous alga devoid of heterocysts (4).

Acetylene reduction assay (ARA) is an indirect, simple method, more sensitive than the ^{15}N and Kjeldahl methods. The relationship between the rate of reduction of C_2H_2 and that of N_2 varies with the experimental conditions and is seldom experimentally determined (5). Bacterial oxidation of ethylene can reduce the ARA values, and anerobic production of ethylene can increase them.

Several modifications have been introduced in sampling procedures and assay chambers for ARA. At ICRISAT, a planted core assay technique has been developed for field-grown millet and sorghum plants, taking into consideration the factors responsible for low and variable nitrogenase activity estimated by the soil-core assay (6). Similarly, tube and pot assay techniques have been developed for intact plant assays (7). Lines of chickpea, groundnut, pigeonpea, pearl millet, and sorghum have been screened for N_2-ase activity, using ARA techniques (7, 8). Diurnal and seasonal variations in N_2-ase activity of groundnut, pearl millet, and sor-

ghum were recorded (6, 9) and effects of light, temperature, moisture, and combined N on N_2-ase activity of these crops have been studied (7, 9). Using ARA, N_2-fixation by chickpea cv K850 has been estimated as 2 kg N/ha/season at ICRISAT Centre and 41 kg N/ha/season at Hisar (10). Despite the limitations of the ARA technique, it will be the preferred technique for the study of N_2-ase activity because of its simplicity and high sensitivity. Its use should be restricted to qualitative and comparative evaluations and certain precautions should be followed. ARA is a short-term measurement of the enzyme activity, and the effects of various environmental and other factors affecting ARA make its quantification and extrapolation to total N_2 fixed over a growing season questionable.

Methods based on N analysis are more reliable and direct measure of BNF, but their main limitation is low sensitivity. Several approaches using N analysis namely, increment in N accretion in plants grown in N-free conditions, nitrogen balance studies, and difference method, have been used for BNF measurements. Pot culture studies with finger millet, forage grasses, paddy, pearl millet, sorghum have shown positive N balances from inoculation; they have been used to study the effect of factors like combined N on BNF (7, 11). Reported estimates of BNF with cereals and grasses, based on N balance in pots and fields, have varied from 11-148 kg N/ha (12). Such experiments are difficult to conduct under field conditions; they need to continue for more than one season and require rigorous soil sampling.

The Kjeldahl or dumas methods estimate total nitrogen in a sample; they do not distinguish between N obtained from BNF and N obtained from other sources. With legumes, attempts have been made to account for N for other sources than N_2, using noninoculated or nonnodulating legume or cereal controls (13, 14). The basic assumption in using the difference method is that N_2 fixing and reference plants have similar seasonal patterns of soil N uptake. Thus, if the yield of the test crop exceeds that of the reference plant, the difference is attributed to N_2-fixation. However, increased total plant N may be from other factors, such as increased efficiency of fertilizer or soil N use from alteration of rooting habits, in addition to BNF. Pigeonpea cultivars of different maturity fixed 4-69 kg N/ha using long-duration sorghum, and nodulated groundnut cultivars fixed 178-184 kg N/ha per season using sorghum or nonnodulating groundnut as reference crops (8, 15). With soybeans, estimated N_2 fixation (167 and 198 kg N/ha at two sites) was the same using the difference and isotope dilution methods (16), whereas in other comparison studies difference method gave lower estimates of BNF and was found consistently less precise than the isotope dilution technique (17).

The stable, heavy isotope of nitrogen (^{15}N) is used as a tracer in BNF investigations. In direct $^{15}N_2$ incorporation studies, plants are exposed to $^{15}N_2$ and after incubation, plant tissues are examined for the concentrations of the heavy isotope. Such studies with cereals and tropical grasses using $^{15}N_2$ have been summarized (12). This method is a direct measure of BNF and also provides information on the amount of fixed N transferred to the host in associative N_2 fixation or from fixing plant to nonfixing plant in intercropped systems. This method is not relevant to field experiments because of the difficulty, in enclosing big plants in air-tight containers a large quantity of $^{15}N_2$ is required. It can be used to establish the ratio of ARA to N_2 fixation in the systems. The isotope (^{15}N) dilution technique gives integrated estimates of N_2 fixation over the growing season in field or pot studies. In a critical review of the use of isotope dilution technique in BNF estimations (14), it has been emphasized that this approach to measuring N_2 fixation is based on several assumptions, the validity of which has not often been established. Evidence is accumulating to suggest that errors in such estimation may be greater than is generally believed. Labeling techniques, such as the chemical and physical form, ^{15}N enrichment, time, rate, and method of application of the isotope have significant effect on estimates of N_2 fixation. The selection of proper reference plant also has a significant effect on estimates of N_2 fixation. Available literature indicates significantly different estimates of N_2 fixed from using different reference plants. The requirement for an ideal reference plant when faced with declining ^{15}N enrichment in the soil has been well described (18). The use of treatments which stabilize ^{15}N enrichment in the plant's available soil N has also been recommended (18). Pigeonpea plants derived 86% of their N in plant tops (66 kg N/ha), in compared with 0% in a long-duration sorghum cultivar (15). In pot studies, lines of sorghum and pearl millet varied in their N_2-fixing potential, and as much as 27% of total plant N in sorghum line IS 801 was derived from BNF in, compared with 0% in IS 3003.

The "A value" method has been used (19) to estimate N inputs through BNF. As "A value" expression is dependent on fertilizer addition rates and fertilizer N recovery by the fixing plant, errors can be introduced by fertilizer N losses. Several studies have shown that "A values" for reference plants do vary with increasing rates of N additions, but the results have not been consistent (14).

References :

1. Hill, S., Postgate, J. R. (1969) Gen. Microbiol. **58 :** 277.
2. Postgate, J. R. (1971) Gen. Microbiol. **21 :** 287.

3. Watanabe, I & Barraquio, W L (1979) Nature 277 : 56'

4. Stewart, W. D. P. (1971) Plant and Soil (Spl. vol.) 377.

5. Knowles, R. (1981) In : Current perspectives in Nitrogen Fixation, A. H Gibson and E Newton (eds.) Academy of Science, Canberra, p. 327.

6. Wani, S. P., Dart, P J. & Upadhyaya, M N. (1983) Can. J Microbiol. 29 : 1063.

7. Wani, S. P. (1985) In : New Trends in Biological Nitrogen Fixation, N. S. Subba Rao (ed.), Oxford-IBH, New Delhi, (in press).

8. Nambiar, P. T. C., Rupela, O. P. & Kumar Rao, J. V. D K. (1985) In : New Trends in Biological Nitrogen Fixation, N. S. Subba Rao (ed.), Oxford-IBH, New Delhi (in press).

9. Nambiar, P. T. C. & Dart, P. J. (1983) Peanut Sci. 10 : 26.

10. Thompson, J. A., Kumar Rao J. V. D K., Rupela, O. P., Giller, K. E. & Day, J. M. (1984) In : Proceedings of Nifial Conference on Nitrogen Recycling in Cropping Systems Involving Legumes (in press).

11. App, A., Bouldin, D. R., Dart, P. J. & Watanabe, I. (1980) In : Soil Related Constraints to Food Production in the Tropics, International Rice Research Institute, Manila, Philippines. p. 319.

12. Wani, S. P. (1986) In : Proceedings of Working Group Meeting on Cereal Nitrogen Fixation, ICRISAT, Patancheru, India (in press).

13. Rennie, R. J. (1979). Revue d' Ecologie et de Biologie du Sol. 16 : 455.

14. Chalk, P. M. (1985) Soil Biol. Biochem. 17 : 389.

15. Kumar Rao, J. V. D. K., Dart, P. J., Matsumoto, T. & Day, J. M. (1980) In : Proceedings of the International Workshop on Pigeonpeas, ICRISAT, Patancheru, India p. 190.

16. Vasilas, B. L. & Ham, G. E. (1984) Agron. Jour. 76 : 759.

17. Rennie, R. J. (1984) Agron. Jour. 76 : 785

18. Witty, J. F. (1983) Soil Biol. Biochem. 15 : 631.

19. Fried, M. & Broeshart, H. (1975) Plant and Soil. 43 : 707.