Biol Fertil Soils (1987) 5:23–25

Biology and Fertility of Soils

A comparison of nitrogen fixation in genotypes of groundnut (*Arachis hypogaea* L.) using ^{15}N-isotope dilution*

K. E. Giller[1], P. T. C. Nambiar[2], B. Srinivasa Rao[2], P. J. Dart[1]*** and J. M. Day[1]**

[1] Soil Microbiology Department, Rothamsted Experimental Station, Harpenden, AL5 2JQ, UK
[2] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P. O., 502324, Andhra Pradesh, India

Summary. Nitrogen fixation in seven groundnut genotypes was measured by ^{15}N-isotope dilution using a non-nodulating cultivar of groundnut as the non-fixing reference plant. Nitrogen fixation varied between 100 kg N ha^{-1} in genotype J-11 and 153 kg N ha^{-1} in Robut 33-1. The amount of plant-available soil N was small, so that 86% – 92% of plant nitrogen was derived from N_2-fixation. Thus differences in N_2-fixation between genotypes closely reflected differences in their total N accumulation.

Key words: N_2-fixation – *Arachis hypogaea* – ^{15}N isotope dilution – Groundnut – *Rhizobium* inoculation – Plant available soil N

Groundnut (*Arachis hypogaea* L.) is often grown in soils poor in nitrogen, but responds little to added fertilizer. Substantial variation between genotypes of groundnut in nodulation and acetylene reduction activity has been found (Arunachalam et al. 1978; Elkan et al. 1980; Nambiar and Dart 1980), but the amounts of nitrogen fixed in the field have not been determined using ^{15}N-labelled fertilizer.

In work described here, the ^{15}N-isotope dilution method has been used to estimate the amount of N_2 fixed by several groundnut genotypes in the field. A non-nodulating groundnut genotype was used as a control, as this was considered more likely to match the rooting and nitrogen accumulation patterns of the nodulated genotypes than non-legume crops, and thus reduce possible errors (Witty 1983). Growth of the non-nodulating groundnut is poor in the absence of a large supply of available N (ICRISAT 1981), and N fertilizer was applied to ensure reasonable growth of the control crop.

Materials and methods

The experiment was carried out at the ICRISAT centre, Patancheru, A. P., India. A cover crop of *Zea mays* L. was grown (and above-ground plant parts removed) in the previous season to deplete the available N in the Alfisol soil. The experiment was sown on 2. 12. 81 with six replicate blocks (13.5 × 18 m). The control treatment of the non-nodulating groundnut, fertilized with 100 kg N ha^{-1}, was located centrally in each block and other treatments randomized around it in order to maintain the closest proximity possible between each treatment and the control. The N_2-fixing treatments were cultivars J11, Gangapuri (Spanish types), PI 259747 (Valencia type), NC 17, ICGS22 and Robut 33-1 (Virginia types), non-inoculated, and Robut 33-1 inoculated with *Rhizobium* strain NC92, all fertilized with 10 kg N ha^{-1}. A further treatment, in which the non-nodulating groundnut was grown with 200 kg N ha^{-1}, was included to check if the rate of nitrogen addition had an effect on the estimate of the available soil-N pool. Seeds were spaced at 10-cm intervals within 75-cm rows and treatment plots consisted of six rows of 6 m (27 m^2).

All fertilizer was applied as ammonium sulphate, with ^{15}N-labelled fertilizer applied to central subplots (4.5 m^2) of each treatment with enrichments of 5.123 atom % ^{15}N excess (10 kg N ha^{-1}), 0.512 atom % ^{15}N excess (100 kg N ha^{-1}) and 0.256 atom % ^{15}N excess (200 kg N ha^{-1}). The experiment was irrigated at sowing and as required (about 10- to 15-day intervals), using Perfor overhead irrigation until the plants were 39 days old, and subsequently furrow irrigation was employed.

All plots were harvested 89 days after sowing. Two metres of each of the central two rows of the ^{15}N subplots (3 m^2) were removed for chemical analysis, and a further sample from 14.25 m^2 was taken for yield and dry matter estimates. Fresh plant material was weighed on site and a subsample taken to estimate water con-

* ICRISAT Journal Article no. 600

Present addresses:

** Department of Biochemistry, Physiology and Soil Science, Wye College, University of London, Wye, Ashford, Kent, TN25 5AH, UK

*** Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra, ACT2601, Australia

Offprint requests to: K. E. Giller

Table 1. N_2-fixation by groundnut cultivars estimated by isotope dilution using a non-nodulating genotype (100 kg N ha^{-1}) as a reference plant

Treatment	Total dry matter (kg ha^{-1})	Pod yield (kg ha^{-1})	Total N (kg ha^{-1})	Atom % ^{15}N excess	% Fertilizer recovery	N from soil (kg ha^{-1})	N from fixation (kg ha^{-1})	% N fixed
Robut 33-1 × NC-92	5870	3000	165	0.028	7.2	12	152	92
Robut 33-1	5630	2990	162	0.024	7.3	12	149	92
NC-17	5200	2400	150	0.031	9.0	16	133	89
ICGS-22	4910	2230	127	0.023	5.6	10	117	92
Gangapuri	4790	2290	131	0.032	8.2	14	116	89
PI-259747	5250	1960	126	0.039	9.7	16	109	86
J-11	4330	1830	111	0.029	6.1	11	100	90
Non-nod 100 kg N ha^{-1}	2570	920	41	0.193	15.6	25	0[a]	0[a]
Non-nod 200 kg N ha^{-1}	3480	1120	60	0.137	16.0	27	1.2	1.2[a]
SE	148	95	4.5	0.0028	0.99	1.53	4.8	1.0

Apparent soil N pool (A-value) estimated to be 163 kg ha^{-1} using the non-nod × 100 kg N ha^{-1} treatment and 175 kg ha^{-1} using the non-nod × 200 kg N ha^{-1} treatment

[a] Values excluded from statistical calculations

tent. Plants sampled from N subplots were separated into shoot, husk and seed fractions for analysis. Nitrogen content was determined by Kjeldahl digestion and by an automated indophenol blue method (Anon 1978). Petroleum ether (BP 40 °C) was used to remove excess oil from the seed before digestion. Nitrogen in the digests was concentrated by a Conway microdiffusion technique (Conway 1939) and ^{15}N enrichments measured using a Micromass 622 mass spectrometer (VG Isogas, Norwich, Cheshire, UK). Amounts of nitrogen fixed were calculated using equations given by Witty (1983).

Results and discussion

The non-nodulating groundnut produced 1000 kg ha^{-1} more dry matter with 200 kg N ha^{-1} than with 100 kg N (Table 1). Although the two control crops accumulated different amounts of nitrogen, estimates of the apparent soil N pool calculated from their ^{15}N enrichments were similar. Fertilizer recovery (%) and the amount of soil nitrogen taken up were not significantly different between the two fertilizer treatments of the non-nodulating groundnut.

Fertilizer recovery (%) was less in all nodulated groundnut lines than in the control treatments. Estimates of nitrogen fixed ranged from 153 kg ha^{-1} in Robut 33-1 (the recommended cultivar for cultivation in Andhra Pradesh) to 100 kg ha^{-1} in J11. In this trial, inoculation of Robut 33-1 with cowpea *Rhizobium* strain NC92, a combination often effective in increasing yields (Nambiar et al. 1982), did not increase the yield significantly above that of uninoculated Robut 33-1. Lack of a yield increase was probably due to NC92 having been used as an inoculum in this field previously. Of the other groundnut genotypes tested here, NC17 was the only line which fixed a similar amount of nitrogen to Robut 33-1. The ranking of genotypes confirms earlier findings that Virginia types generally fix more nitrogen that Valencia or Spanish types (Nambiar et al. 1980).

Nodulated groundnut genotypes absorbed only a small proportion of the applied nitrogen and there was some small, but significant, variation between genotypes in percentage fertilizer recovery (overall mean 7.6% ± 0.52%). With the irrigation methods employed, it is unlikely that the low fertilizer recovery was due to leaching losses of the fertilizer; it probably reflects a poor ability of groundnut to absorb fertilizer (Nambiar et al. 1986). Non-nodulating groundnut grown without added nitrogen only accumulates 20 – 30 kg N ha^{-1}, and such genotypes require at least 300 kg N ha^{-1} in fertilizer to produce maximal yield (ICRISAT 1982). If the soil nitrogen pool is less than 200 kg N ha^{-1}, as in this soil which is typical of those where groundnut is grown, nodulated genotypes would rarely derive more than 16 kg N ha^{-1} from the soil. Indeed sorghum (*Sorghum bicolor* (L.) Moench), which has a deeper, more extensive rooting system, typically removes only 30 – 55 kg ha^{-1} soil nitrogen when grown on alfisol soils (ICRISAT 1982). As groundnut crops commonly accumulate more than 100 kg N ha^{-1}, they therefore contain a relatively small contribution from the soil and a high proportion of plant N from fixation (a range of 86% – 92% N fixed; Table 1). Hence, when groundnut is grown without fertilizer N, the total nitrogen accumulation is likely to be a good indication of the plant benefit from N_2-fixation.

References

Anon (1978) Analysis of crops, soils and fertilizers. Soil and Plant Nutrition Department, Rothamsted Experimental Station, Harpenden, England

Arunachalam V, Pungle GD, Dutta M, Nambiar PTC, Dart PJ (1984) Efficiency of nitrogenase activity and nodule mass in predicting the relative performance of genotypes assessed by a number of characters in groundnut (*Arachis hypogaea*). Exp Agric 20:303 – 310

Conway EJ (1939) Microdiffusion analysis and volumetric error. Crosby Lockwood, London

Elkan GH, Wynne JC, Schneeweis JJ, Isleib TG (1980) Nodulation and nitrogenase activity of peanuts isolated with single strain isolates of *Rhizobium*. Peanut Sci 7:95 – 97

ICRISAT (1981) Annual report. ICRISAT, Patancheru A. P., India, p 187

ICRISAT (1982) Annual report. ICRISAT, Patancheru A. P., India, p 249

Nambiar PTC, Rego TJ, Srinivasa Rao B (1986) Comparison of the requirements and utilization of nitrogen by genotypes of sorghum (*Sorghum bicolor* (L.) Moench), and nodulating and non-nodulating groundnut (*Arachis hypogaea* L.). Field Crops Res 15:165 – 179

Nambiar PTC, Dart PJ (1980) Studies on nitrogen fixation in groundnut at ICRISAT. In: Gibbons RW (ed) Proceedings of the International Symposium on the Groundnut, ICRISAT, Patancheru A. P., India, pp 110 – 124

Nambiar PTCN, Dart PJ, Srinivasa Rao B, Ravi-Shankar HN (1982) Response of groundnut (*Arachis hypogaea*) to inoculation. In: Graham PH, Harris S (eds) Biological nitrogen fixation technology for tropical agriculture. CIAT, Cali, Colombia, pp 241 – 248

Witty JF (1983) Estimating N_2-fixation in the field using ^{15}N-labelled fertilizer: some problems and solutions. Soil Biol Biochem 15:631 – 639

Received July 24, 1986