Ontogeny and nitrogen fixation in mungbean, blackgram, cowpea, and groundnut

R. Senaratne, D.S. Ratnasinghe

Department of Agronomy, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

Received: 15 April 1992

Abstract. Ontogenic variations in N₂ fixation and accumulation of N by the mungbean (Vigna radiata L. Wilczek), blackgram (Vigna mungo L. Hepper), cowpea (Vigna unguiculata L. Walp.), and groundnut (Arachis hypogaea L.) were studied by a ¹⁵N-dilution technique. Pots filled with 7 kg of red yellow podzolic soil were used. Samples were taken 20, 40, 60, and 80 days after emergence which approximately corresponded to preflowering, flowering, early/mid-pod filling and late pod filling stages, respectively. During early growth (up to 40 days after emergence), the carryover of seed N accounted for a considerable fraction of the total plant N in the legumes, the highest being in the groundnut. With a correction for carryover, the groundnut derived over 45% of its N content from the atmosphere 20 days after emergence whereas the corresponding figures were 33% for the blackgram and about 28% for the cowpea and mungbean. Between flowering and early pod fill, there was a rapid increase in N₂ fixation in all legumes except in groundnut which showed highest fixation from 60 to 80 days after emergence. In the mungbean, N₂ fixation and uptake of soil N were insignificant 60 days after emergence while in other legumes these processes continued beyond this time. All legumes derived about 90% of their N from atmosphere by 80 days after emergence. However, due to considerable interspecific differences in total N yield the final amount of N₂ fixed showed an appreciable variation among legumes. It was highest in the groundnut (443 mg N plant⁻¹) followed by the cowpea (385), blackgram (273), and mungbean (145), respectively. The groundnut maintained nodules until the late pod filling stage while in other legumes, nodules senesced progressively following the mid-pod filling stage. During pod filling there was a net mobilization of N from vegetative tissues to developing pods in the mungbean, which amounted to about 20% of N in seeds. This mobilization was not evident in other legumes.

Key words: N₂ fixation – Ontogeny – ¹⁵N – Vigna radiata – Vigna mungo – Vigna unguiculata – Arachis hypogaea

Correspondence to: R. Senaratne

Legumes are able to derive a considerable proportion of their N requirement from the atmosphere via symbiotic N₂ fixation; the proportion depends on host genotype, rhizobial strain, environment and their interactions. Under favorable conditions nodules appear on primary roots of the mungbean and blackgram within 1 week of emergence (Lawn and Ahn 1985) and measurable acetylene reduction activity can be detected within 10–25 days of sowing (Lawn and Ahn 1985). Similar observations have been made in the groundnut (Nambiar et al. 1988) and the cowpea (Senanayake et al. 1987). In the mungbean, nodule numbers and mass increase rapidly during the vegetative phase, reach a maximum at flowering or during early pod filling stage (Lawn and Ahn 1985), and decline rapidly thereafter with the onset of nodule senescence. The decline in symbiotic performance following pod development has been attributed to changes in source-sink relationships, resulting in a decreased supply of carbohydrates to the nodules (Lawn and Brun 1974; Hardy and Havelka 1976). A comparable ontogenic profile in the number and mass of nodules has been observed in the cowpea (Eaglesham et al. 1977). In contrast, the groundnut can support active nodules, and thus N₂ fixation, until just before harvest (ICRISAT 1978; Sung and Sun 1990; Senaratne and Edirimanna unpublished data 1991).

Legumes such as the cowpea, groundnut, mungbean, and blackgram are important food legumes in the tropics and subtropics, and increasing attention is being paid to improving the N₂ fixation and N-residual effect of these legumes in an attempt to develop sustainable cropping systems. There is a dearth of reliable estimates on N₂ fixation by these legumes and hardly any quantitative information is available on the time-course of their symbiotic performance. Some studies on ontogenic variations in nitrogenase activity have been carried out using the acetylene reduction assay, in the groundnut (Nambiar and Dart 1983; Sung and Sun 1990), mungbean (AVRDC 1978), and cowpea (Senanayake et al. 1987). However, this assay gives only a short-term kinetic measurement and has serious limitations in determining N₂ fixation by legumes (Knowles 1981). A time-course study of N₂ fixation using ¹⁵N techniques, besides affording an integrat-
ed value of N₂ fixation over a given period of time, would allow a determination of the relative N contribution by the legumes and the relative importance of soil, fertilizer, and atmosphere to the N economy of the legumes at various stages of plant growth. This information could be useful in developing management practices, including the application of fertilizer, to increase legume yields by optimizing N₂ fixation and N nutrition. Accordingly, there have been several studies on the time-course of N₂ fixation by legumes such as the soybean (Zapata et al. 1987a; Herridge and Bergersen 1988), field bean (Rennie and Kemp 1984; Herridge and Bergersen 1988), and faba bean (Brunner and Zapata 1984; Zapata et al. 1987b). However, no previous studies have been reported on tropical legumes such as the cowpea, groundnut, mungbean, and blackgram. We therefore undertook ¹⁵N studies to ascertain the ontogenic variation in N₂ fixation of the above legumes.

Materials and methods

The experiment was conducted outdoors during the period of June to August 1988 at the Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka. The legume seeds were inoculated with appropriate peat-based inocula received from the Biological Nitrogen Fixation Regional Centre in Bangkok. Just before the seeds were sown, all the pots were supplied with labelled ammonium sulphate of 5~15N atom excess at the rate of 25 mg kg⁻¹ soil each. Upon germination, the legume plants were thinned to three per pot and the P. miliaceum (Panicum miliaceum L.), which has an N-uptake pattern similar to those of the above legumes, was planted as a reference crop to determine N₂ fixation by a ¹⁵N method (Fried and Middleboe 1977).

The legume seeds were inoculated with appropriate peat-based inocula received from the Biological Nitrogen Fixation Regional Centre in Bangkok. Just before the seeds were sown, all the pots were supplied with labelled ammonium sulphate of 5% ¹⁵N atom excess at the rate of 10 mg N kg⁻¹ soil (equivalent to about 20 kg N ha⁻¹), as an aqueous solution and mixed thoroughly with the soil. P and K were applied at a rate of 25 mg kg⁻¹ soil each. Upon germination, the legume plants were thinned to three per pot and the P. miliaceum plants to six per pot. The experiment was arranged in a randomized complete block design with five replicates and provision for four sequential samplings.

Watering, weeding, and spraying against pests and diseases were done when necessary. Four samples were taken 20, 40, 60, and 80 days after emergence, which approximately corresponded to preflowering, flowering, early/mid-pod filling and late pod filling stages, respectively. During sampling care was taken to minimize damage to the root system, and the number and fresh weight of nodules were recorded. Above-ground material was separated into pods and stover (when applicable), and the number and fresh weight of nodules were recorded. A fresh weight of groundnut appeared 40 days after emergence, which corresponded to the early to mid-pod filling stage of the crops. Beyond 60 days after emergence, there was a marked decline in nodule mass. The highest nodule mass for the groundnut appeared 40 days after emergence. This legume also maintained nodules until 80 days after emergence, which was not observed in the other legumes. The number of nodules on the groundnut decreased between 60 and 80 days after emergence. However, the fresh weight per nodule increased from 3.3 to 6.8 mg during this period, which may have been due to a new flush of nodules by 80 days after emergence.

Atom% ¹⁵N excess

The atom% ¹⁵N excess in all the legumes was significantly lower than in the reference crop at all sampling stages (Table 2). This indicates that a significant proportion of N was derived by the legumes from the atmosphere. In the groundnut the atom% ¹⁵N excess was significantly lower 20 days after emergence than that in the other legumes. The fresh weight per nodule increased from 3.3 to 6.8 mg during this period, which may have been due to a new flush of nodules by 80 days after emergence.

Sources of plant N and N accumulation

During the early stages of growth (20 days after emergence), seed N accounted for a considerable proportion of total N in the legumes, reaching 25% in the groundnut and 15% in the other legumes. Thus in addition to N₂

Table 1. Ontogenic variation in number and fresh weight of nodules in mungbean, blackgram, cowpea, and groundnut

<table>
<thead>
<tr>
<th>Crop</th>
<th>20 DAE</th>
<th>40 DAE</th>
<th>60 DAE</th>
<th>80 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nodule number</td>
<td>Nodule fresh weight (mg plant⁻¹)</td>
<td>Nodule number</td>
<td>Nodule fresh weight (mg plant⁻¹)</td>
</tr>
<tr>
<td>Mungbean</td>
<td>07</td>
<td>16.5</td>
<td>31</td>
<td>685.4</td>
</tr>
<tr>
<td>Blackgram</td>
<td>12</td>
<td>20.5</td>
<td>63</td>
<td>742.6</td>
</tr>
<tr>
<td>Cowpea</td>
<td>13</td>
<td>73.6</td>
<td>72</td>
<td>977.9</td>
</tr>
<tr>
<td>Groundnut</td>
<td>41</td>
<td>80.6</td>
<td>136</td>
<td>1123.0</td>
</tr>
</tbody>
</table>

DAE, Days after emergence

(Fiedler and Proksch 1975). The percentage and amount of N derived from the atmosphere were calculated by a ¹⁵N method (Fried and Middleboe 1977).

Results

Nodulation

The groundnut showed the earliest nodulation and had over 40 nodules per plant by 20 days after emergence while the corresponding figures for the cowpea and blackgram were around 12 with 7 for the mungbean (Table 1). Thus, the mungbean was late in nodulating compared with the other three legumes. The groundnut maintained the highest number of nodules per plant throughout the experiment. However, the fresh weight of groundnut was greater than that of the other legumes only until 40 days after emergence, and thereafter the cowpea was superior to the groundnut in this respect (Table 1). Except for the groundnut, all other legumes showed the maximum number and weight of nodules 60 days after emergence, which corresponded to the early to mid-pod filling stage of the crops. Beyond 60 days after emergence, there was a marked decline in nodule mass. The highest nodule mass for the groundnut appeared 40 days after emergence. This legume also maintained nodules until 80 days after emergence, which was not observed in the other legumes. The number of nodules on the groundnut decreased between 60 and 80 days after emergence. However, the fresh weight per nodule increased from 3.3 to 6.8 mg during this period, which may have been due to a new flush of nodules by 80 days after emergence.

Atom% ¹⁵N excess

The atom% ¹⁵N excess in all the legumes was significantly lower than in the reference crop at all sampling stages (Table 2). This indicates that a significant proportion of N was derived by the legumes from the atmosphere. In the groundnut the atom% ¹⁵N excess was significantly lower 20 days after emergence than that in the other legumes.

Sources of plant N and N accumulation

During the early stages of growth (20 days after emergence), seed N accounted for a considerable proportion of total N in the legumes, reaching 25% in the groundnut and 15% in the other legumes. Thus in addition to N₂