Exploration of the Nitrogen Transport System of a Nodulated Legume Using $^{15}$N

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Summary. Feeding experiments using $^{15}$N$_2$ or $^{15}$NO$_3$ are described investigating the transport of nitrogen in the field pea (Pisum arvense L.). Nitrogen assimilated by root or nodules moves preferentially upwards to the shoot through the xylem. Parts of the root below or distal to a region of assimilation can benefit from this nitrogen but do so to a much greater extent when the shoot is left attached than when it has been removed. A considerable proportion of the nitrogen received by a shoot from the root or nodules is apparently returned to the root in the translocation stream, this "cycled" nitrogen being especially important in the nutrition of outlying parts of nodulated roots growing in media lacking combined nitrogen.

Nitrogen from nitrate fed to a mature leaf is exported in quantity to all parts of the plant except older regions of the shoot. Leaf and stem segments immediately above the fed leaf, and the root and its nodules receive large shares of this nitrogen, although the root's share declines noticeably as the plant ages.

The root appears to be extremely inactive in transferring nitrogen from the downward translocation stream across to the stream of nitrogen leaving the root in the xylem. This may act as a major obstacle to the free circulation and mixing of nitrogen within the plant body.

A scheme is proposed embracing the main quantitative features of the transport system for nitrogen in the species.

Introduction

Earlier reports on the nitrogen metabolism of the pea plant have described the location of its main centres for assimilation of inorganic nitrogen, the nitrogenous compounds which it synthesizes at these centres, and the relative significance of each of its main nitrogen-containing solutes in transport and storage of nitrogen and in the synthesis of protein (see Pate, 1968).

Extensive use was made in the above investigations of experiments studying the fate of specific amino compounds fed to the plant in $^{14}$C-labelled form or formed in shoot, nodulated root and root bleeding sap after $^{14}$CO$_2$ had been assimilated by the leaves. Findings relevant to the metabolism and movement of nitrogenous compounds were obtained, but since only the carbon of the molecules involved could be...
traced, the studies failed to determine rates of assimilation and turnover of nitrogen as such, and to ascertain how freely this element moves within and between the various transport channels of the nodulated plant. The experiments described here using isotopic nitrogen attempt to make good some of these deficiencies.

Methods

a) Plant Culture

Plants of field pea (*Pisum arvense* L., cv. “Black-eyed Susan”), grown in water or sand culture and nodulated with effective *Rhizobium* strain V5 were used for the experiments. Plant culture took place in a Sherer-Gillett growth cabinet (Model CL 512), the standard environmental conditions being 18°C day (16 h, 27000 lux), 10°C night (8 h).

b) Administration of Isotopic Nitrogen

(i) Feeding $^{15}$N$_2$ to Roots. Well-nodulated 5-leaf plants grown in water culture lacking inorganic nitrogen were selected and the root of each plant transferred carefully to a 500 ml flask half-filled with minus-nitrogen culture solution. The epicotyl was sealed into the neck of the flask with plasticene. The upper nodulated parts of the root were then exposed to a gas mixture consisting of 20% O$_2$, 80% N$_2$ (containing $^{15}$N at 95 atom % excess), the lower parts of the root remaining immersed in the culture solution. The gas space around the roots was replenished frequently to avoid depletion of oxygen and build up of carbon dioxide, and treatment with the isotope continued for the first day of the experiment. For the next two days the upper parts of the roots were exposed to air (unlabelled). The plants were then harvested.

(ii) Feeding $^{15}$NO$_3$ to Part of a Divided Root System. 5-leaf, well nodulated plants grown in minus-nitrogen culture solution were used. The root of each was transferred to a double culture vessel so that the upper lateral roots could be exposed to culture solution containing labelled nitrate (140 p.p.m. NO$_3$—N as KNO$_3$ at 34.7 atom % excess $^{15}$N), while the lower parts of the root continued to receive minus nitrogen culture solution. After three days of such treatment the plants were harvested.

(iii) Feeding $^{15}$NO$_3$ to Leaves. Nodulated plants were grown in sand watered with culture solution lacking inorganic nitrogen, and at the correct plant age a particular leaf on a plant was fed with nitrate. The standard procedure was to start supplying the selected leaf just as it became fully expanded and presumably active in export. Each day for a week it was immersed for seven hours of the photoperiod in a solution of K$^{15}$NO$_3$ containing 210 p.p.m. NO$_3$—N at an enrichment of 95 atom % excess $^{15}$N, excess nitrate being removed from its surface at the end of each day’s feeding and especial care being taken to avoid contamination of other parts of the plant with the labelled nitrate. (Solution feeding proved to be a much more successful method of promoting uptake of nitrogen by a pea leaf than did foliar spraying. Also since the leaf was illuminated throughout its daily immersion in the labelled nitrate, it was unlikely that damaging anaerobic conditions would prevail during periods of feeding, while it was possible that photosynthetically-produced reductant might foster the assimilation of absorbed nitrate.)