NITROGEN FIXATION IN THE ROOT NODULES OF Vicia Faba L. IN RELATION TO THE ASSIMILATION OF CARBON

I. PLANT GROWTH AND METABOLISM OF PHOTOSYNTHETIC ASSIMILATES

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SUMMARY

Nitrogenase activity of the root nodules of field-grown Vicia faba increased during plant growth until 3-4 weeks after the onset of flowering. The subsequent decline in activity was never greater than to 20% of the maximum, until finally plants were killed by frost. Nodules of medium size (100-200 mg fresh weight) showed greatest nitrogenase activity.

After photosynthesis in $^{14}$CO$_2$ for 30 min, maximum accumulation of $^{14}$C-assimilates in the nodules occurred within 90 min of synthesis. The relatively low levels of radioactivity associated with the organic acid fraction of the nodules, the absence of sucrose which is present in large quantities in the roots and the appearance of radioactivity in the nodule amino acids within 30 min of feeding the shoot $^{14}$CO$_2$ show that photosynthates are rapidly metabolized on arrival in the nodules. The distribution of radioactivity among the constituents of the basic fraction confirms the importance of glutamate, glutamine and aspartate as early products of the metabolism of the fixed nitrogen in broad bean nodules receiving ample supplies of new photosynthates.

INTRODUCTION

The necessity for a continuing supply of photosynthates to the nodules for the maintenance of maximum nitrogenase activity has been shown for several species (Virtanen, Moisio and Burriss, 1955; Wheeler, 1971; Lawrie and Wheeler, 1973) although a number of factors, such as the age and height of a species and the presence of substantial food reserves, may influence the effect of a change in the supply of photosynthates (Wheeler and Lawrie, 1975). The high invertase activities reported in nodules of serradella (Kidby, 1966) and lupin (Robertson and Taylor, 1973) suggest that sucrose translocated from the shoot is rapidly utilized to support the metabolic activities of the nodule tissues. Bach, Magee and Burriss (1958) studied the metabolism of $^{14}$C-photosynthates translocated to the nodules of field-grown soybean plants after photosynthesis of the shoots in $^{14}$CO$_2$ for several hours. Ion-exchange chromatography of ethanolic extracts of plants harvested in the evening (‘day’ plants) or the following morning (‘night’ plants) showed that 60% of the radioactivity of the nodules was in the neutral fraction, mainly as glucose and fructose. Sucrose was abundant in the roots but was not detected in the nodules, probably due to high invertase activity. The basic fraction contained twice the

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roots was much lower but showed similar changes to the nodules, while the specific activity of the leaves after 5.5 h had declined to one quarter of its value immediately after exposure to $^{14}\text{CO}_2$.

![Graph]

Fig. 1. Changes during growth of plants in acetylene reduction by detached nodules from crops planted on 1 July (●) and 15 July (■). Each point represents the mean of six samples.

In nodule extracts, the neutral fraction contained more than 70% of the total radioactivity of ethanolic extracts at all times with the maximum radioactivity achieved after 1.5 h ‘cold chase’ (Fig. 2b). Glucose and fructose were the only sugars detected, containing between them 87% of the total radioactivity of chromatograms of this fraction. The remaining 13% occurred as a ‘streak’ in the vicinity of fructose. Sucrose was not detected though it was the main sugar in root extracts.

The acidic fraction accumulated not more than 2% of the total activity of ethanolic nodule extracts (Fig. 2b). Malic acid was the major acidic component, containing 44–67% of the total radioactivity of the acidic fraction (Fig. 2c).

The specific activity of the basic fraction rose to a maximum after 1.5 h ‘cold chase’ (Fig. 2b), at which time it contained 10% of the total ethanolic radioactivity, and then declined to 5% of the total after 5.5 h. Radioactivity was initially highest in glutamic acid (22.5% of the radioactivity on the chromatograms), aspartic acid (30.7%) and glutamine (29.2%) whereas asparagine (quantitatively the major component of the basic fraction) accumulated less than 10% of the activity (Fig. 2c). Leucine/isoleucine accumulated 13.3% of the activity on the chromatograms after 5.5 h ‘cold chase’. Only glutamine showed a decrease in percentage total activity during the initial 30 min ‘cold chase’.

**DISCUSSION**

Changes in the rate of acetylene reduction during the growth and development of the broad bean plant differed from those previously reported for glasshouse-grown pea plants (Lawrie and Wheeler, 1974). Seed production was inhibitory to activity in both species as shown for soybean (Bond, 1941; Lawn and Brun, 1974), *Pisum arvense*
Fig. 2. (a) Changes in the radioactivity of ethanolic extracts of leaves (▲), roots (■) and nodules (○) after exposure of the shoots of 6-week-old plants to $^{14}$CO$_2$ for 30 min. Each point is the mean of four replicate samples. (b) Changes in the distribution of radioactivity in the neutral (■), basic (▲) and acidic (▼) fractions of ethanolic extracts of nodules after exposure of the shoots to $^{14}$CO$_2$ for 30 min. (c) Changes in the distribution of radioactivity among the main radioactive components of the basic and acidic fractions of nodule extracts. Compounds shown are: aspartate (○), asparagine (●), glutamate (▲), glutamine (▲), leucine/isoleucine (▲) and malate (▼).

(Plate, 1958a) and Vicia sativa (Pate 1958b), but the two species differed both in the stage at which inhibition began and in the degree of inhibition. Whereas, in peas, activity declined when the flower buds opened and continued to decline virtually to zero as the nodules became totally green, the activity of bean nodules declined only when fruit formation was advanced (Fig. 1). Also, the nodules never became totally green and still retained 20% of their maximum activity until the plants were killed by frost. These differences could be due in part to the fact that peas were glasshouse-grown whereas beans were field-grown, but this is unlikely to be the whole explanation since Pate (1958a) observed total greening in nodules of field-grown Pisum arvense after fruiting. The different reproductive patterns in the two species are probably responsible for at least part of the differences observed, since continued flowering and fruiting took place in beans while the pea plants soon died after fruit development. Differences in nitrogenase activity between soybeans with indeterminate and determinate flowering patterns
were observed by Hardy et al. (1971), the former fixing only 10% of their total nitrogen before flowering whereas the latter had fixed 25% of the total nitrogen by this time. The flowering process itself inhibits nitrogen fixation relatively little and inhibition of nitrogenase activity appears to be associated mainly with fruit development, although the degree of inhibition is apparently reduced when growth of the shoot apex continues during fruiting. Lawrie and Wheeler (1974) suggested from studies of the translocation of photosynthates within the pea plant during the reproductive period that changes in the supply of photosynthates to the nodules, coupled with changes in the hormonal balance of the plant, may regulate nitrogenase activity at this time.

The higher nitrogenase activity found in medium rather than small or large nodules shows the importance of uniformity in nodule size in assays with detached nodules (Table 1). This agrees with the results of Aprison, Magee and Burris (1954) and Magee and Burris (1972) for soybean nodules, while Akkermans (1971) noted that alder nodules with lobes 3.5-5.0 mm long were five times more active than those with lobes 1.0-1.5 mm long. The reasons for the dependence of nitrogenase activity on nodule size in broad beans have not been examined. However, Bond (1941) proposed that the decreasing activity in older, large soybean nodules may be due to increasing numbers of inactive, infected cells and this may explain the lower rate of nitrogenase activity in large broad bean nodules, particularly those from older plants, which frequently showed greening at the nodule base. It is likely that a peak of nodule activity is reached in nodules of medium size and age, when a balance is achieved between the numbers of active cells and the numbers of inactive basal cells and immature apical cells with little activity, as discussed by Chen and Thornton (1940).

The rapid increase and subsequent decline in radioactivity of the nodules of plants subsequent to feeding the shoots $^{14}$CO$_2$ for 30 min (Fig. 2a) suggests that maximum amounts of the $^{14}$C-assimilates photosynthesized during this period are translocated to the nodules within 90 min. The rapid utilization of photosynthates newly arrived in the nodules for amino acid biosynthesis is shown by the occurrence of radioactivity in the basic fraction of ethanolic nodule extracts immediately after 30 min exposure of the shoots to $^{14}$CO$_2$ (Fig. 2b).

Further analysis of the basic fraction (Fig. 2c) suggests that the accumulation process was biased towards aspartic acid and glutamine, which showed the highest levels of radioactivity both at this time and after more prolonged 'cold chase'. The high levels of radioactivity associated with glutamine immediately after exposure of the shoots to $^{14}$CO$_2$, coupled with the similar, but opposite, changes in radioactivity of glutamate during the experiment are in agreement with the findings of Kennedy (1966a, b) for seradella nodules that these are the first formed aminated compounds. The lower levels of radioactivity associated with asparagine, despite its quantitative importance in the broad bean nodules, probably reflects its role as the main vehicle for the transport of nitrogen from the nodules (Pate, Gunning and Briarty, 1969).

Little information is available at present on the individual roles of the bacterial and the host nodule tissues in the utilization of photosynthetically derived carbon for nitrogen assimilation in broad bean nodules. It has been suggested (Dunn and Klucas, 1973) that the reactions catalyzed by glutamine synthetase and glutamate synthetase, which show high activity in extracts of soybean bacteroids, could form a major route for ammonia assimilation which would account for the rapid labelling of glutamate and glutamine in experiments with isotopic tracers and which would reduce in importance the role of glutamate dehydrogenase in nitrogen assimilation in legume nodules. How-
ever, Brown and Dilworth (1973) found that glutamine synthetase and glutamate synthetase activities from broad bean and pea nodules were both inadequate to account for NH₃ incorporation from N₂ and that bacteroids of Rhizobium meliloti from lucerne and R. phaseoli from red runner bean do not have glutamate synthetase activity. The contributions of the bacteroid and host nodule tissues to ammonia assimilation into organic form could well be different in nitrogen fixing root nodules from different legumes.

Of the organic acids, only malic acid showed detectable radioactivity. No significance can be attached to the fluctuations in the radioactivity of this compound which were observed during these experiments, but the relatively low levels of activity associated with the organic acids suggest that they must be rapidly aminated in nodules active in nitrogen fixation. The metabolism of the organic acids in broad bean nodules is considered by Lawrie and Wheeler (1975).

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