Interdependence of Nitrogen Nutrition and Photosynthesis in *Pisum sativum* L.

II. HOST PLANT RESPONSE TO NITROGEN FIXATION BY RHIZOBIUM STRAINS

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**ABSTRACT**

Physiological responses to infection by strains of *Rhizobium leguminosarum* which differed in their capacity to reduce N$_2$ were determined in 26-day-old pea plants (*Pisum sativum* L. cv. Alaska) grown under uniform environmental conditions in the absence of combined N. The highest N$_2$ reduction rates, calculated from H$_2$ evolution and C$_2$H$_2$-dependent C$_2$H$_2$ production measurements, were approximately 6-fold greater than the lowest. Higher N$_2$ fixation rates were associated with greater CO$_2$ exchange rates (R$^2 = 0.92$) and carboxylation efficiency (R$^2 = 0.99$). Increases in the apparent relative efficiency of N$_2$ fixation [1-(H$_2$ evolved in air/C$_2$H$_2$ reduced)] (acetider efficiency) were associated with increases in whole-plant N$_2$ fixation efficiency (N$_2$/CO$_2$ reduction ratio) (R$^2 = 0.95$). Whole-plant dry weight and total N content were related by regression analysis (R$^2 = 0.98$); both parameters were enhanced by increased N$_2$ fixation in a manner analogous to previously reported increases caused by greater external applications of NH$_4$+. These data reveal that photosynthetic parameters in genetically uniform host plants grown under identical environmental conditions are affected by N$_2$ fixation characteristics of *Rhizobium* strains which differed in their capacity to reduce N$_2$.

**MATERIALS AND METHODS**

Pea (*P. sativum* L. cv. Alaska) plants were maintained, and photosynthesis, N$_2$ fixation, and N content measurements were made as described previously (2), with the following exceptions. All plants were grown in the absence of combined N, and treatments consisted of eight single plant replications inoculated with *Rhizobium leguminosarum* strains TA101 (obtained originally from J. J. Child, N.R.C. Saskatoon, Sask.), 175G10, 128C53, 92A2, or 92F1 (obtained originally from J. C. Burton, The Nitragin Co., Milwaukee). Nitrogen content, N$_2$ fixation, RE$_3$ (9), CER, CE, and C$_2$H$_2$ reduction data were evaluated by Duncan’s multiple range test (5) to determine if significant differences existed in plants inoculated with different strains. The relationships of N$_2$ fixation to CER, N$_2$ fixation to CE, N$_2$ fixation to N content, N content to dry weight, and RE to N$_2$/CO$_2$ fixation ratio were evaluated by linear and nonlinear regression analyses. Data points were fitted with curves corresponding to the function which was associated with the highest statistical significance. Analyses were based on the negative exponential function (y = a[1−exp−(−y)]) for the first three relationships, on the linear function for the fourth, and on the exponential function (y = ae$^x$) for the last relationship.

**RESULTS AND DISCUSSION**

Symbiotic N$_2$ fixation rates were calculated from C$_2$H$_2$ reduction and H$_2$ evolution data for all *Rhizobium* strains tested in association with Alaska pea (Table I). A comparison of C$_2$H$_2$ reduction and H$_2$ production with the computed N$_2$ fixation values obtained in this experiment supports the concept (9) that C$_2$H$_2$ reduction values can misrepresent N$_2$ fixation in the absence of H$_2$ evolution data from the same root nodules. Of 10 possible comparisons between strains for N$_2$ fixation and RE, only one (128C53-92A2) was not significantly different (P ≤ 0.05) in Duncan’s multiple range test (5). The same procedure, however, revealed that only strain 92F1 differed significantly from the other four strains in C$_2$H$_2$ reduction capacity. One interpretation of these data is that strains 128C53 and 92A2 had a greater rate of calculated N$_2$ fixation than strains TA101 and 175G10 because the former evolved less H$_2$ as reflected in the RE values. Strain 92F1 had enhanced N$_2$ fixation not only because of a greater RE but also because of a greater flux of electrons passed through the nitrogenase complex (greater C$_2$H$_2$ reduction). Thus, there may be at least two genetic mechanisms in *Rhizobium* by which N$_2$ fixation can be enhanced.

Regression analysis of RE and N$_2$/CO$_2$ values in Table I revealed that these data fitted a nonlinear function (y = 0.31 e$^{0.63x}$) with R$^2 = 0.95$ (P ≤ 0.05). This finding suggests that under uniform environmental conditions, such as those used in this study, increases in RE of the microsymbiont were associated with...
increased macrosymbiont efficiency (N₂/CO₂ uptake ratio). This observation contrasts with the situation reported previously for Alaska peas grown under different photosynthetic photon flux densities (4). In the latter case, large decreases in RE were measured in plants grown under increasing photosynthetic photon flux densities while the same plants showed increasing N₂/CO₂ uptake ratios.

Symbiotic N₂ fixation, when based on both C₄H₄ reduction and H₂ evolution data, was related positively with CER (R² = 0.92) and CE (R² = 0.99) as indicated by regression equation y = a (1 - be⁻cx) (Figs. 1 and 2). The fact that CER increased with greater N₂ fixation may be explained by the possibility that more N₂ fixation resulted in larger plants which gave greater values for CER, a whole-plant parameter. The corresponding increase in CE, a parameter determined per unit of leaf area, reflects an increase in the activity, and possibly the quantity, of some or all components of the photosynthetic apparatus due to the increased availability of N. The latter suggestion is possible because CE was determined as a function of internal leaf CO₂ concentration which eliminates potential variations in CER measurements resulting from differing stomatal resistances (1).

Whole-plant N content and plant dry weight were related positively as indicated by regression equation y = 16.49x + 0.3109 (R² = 0.98) (Fig. 3). The capacity of the microsymbiont to provide NH₄⁺ for the host plant, therefore, was reflected directly in host response. Maximum N content in the symbiotic plants (strain 92F1) was about 40 mg of reduced N/plant, an amount which corresponded to that found in plants grown in the absence of Rhizobium under identical conditions except for an external 4 mm NH₄⁺ supply (2). Plants grown with less efficient rhizobial strains had total N contents comparable to nonsymbiotic plants provided with smaller amounts of NH₄⁺. Total plant dry weight, however, was significantly greater (P ≤ .01) in the nonsymbiotic peas grown with 8 and 16 mm NH₄⁺ (2) than in the symbiotic plants (Fig. 3). This difference in dry weight is probably due to stunting (7, 8) of pea seedlings which are N-deficient until the N₂ fixation mechanism becomes fully functional.

Several phenomena actively influenced the results of this study. Data in Figure 3, which integrate all C and N metabolism occurring after seed imbibition, reveal that those plants which benefited from a more active N₂-fixing symbiosis had a greater dry weight 26 days after germination. This fact is a consequence of the interdependence of photosynthesis and N₂ fixation. Although both the N₂/CO₂ uptake ratio (Table I) and whole-plant CER (Fig. 1) increased with greater N₂ fixation, it is apparent that neither symbiotic N₂ fixation nor photosynthetic CO₂ reduction could increase without limits. In Figure 1 data fitted to the function y = 30.57 (1 + 0.73e⁻⁰·²x) (R² = 0.92) suggest that the coefficient

![Graph](https://example.com/graph.png)

**Figure 1.** Relationship between symbiotic N₂ fixation and CO₂ exchange rate (CER) in 26-day-old pea plants inoculated with different strains of Rhizobium. Means ± se were computed from five and three replicates respectively. Annotation adjacent to data points refers to the strain number of R. leguminosarum used as inoculant. Nitrogen fixation was computed as (C₄H₄ reduced - H₂ evolved)/3. Data were fitted to a nonlinear function [y = 30.57(1 + 0.73 e⁻⁰·²x)] with R² = 0.92 (P ≤ .05).

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**Figure 2.** Relationship between symbiotic N₂ fixation and carboxylation efficiency (CE) in 26-day-old peas inoculated with different strains of Rhizobium. Annotation, computations, and plants used were as in Figure 1. Data were fitted to a nonlinear function [y = 0.32(1 + 0.55 e⁻¹·⁰x)] with R² = 0.99 (P ≤ .01).

30.57 (i.e. the CER value approached asymptotically by the function) may represent the limit of the Alaska pea-R. leguminosarum symbiosis for net CO₂ exchange under the experimental
conditions. A similar argument may be applied to the N2 fixation-N assimilation relationship in 26-day-old peas in this study (Fig. 4). Here N assimilation (measured by N content) could be limiting. The manner in which pea plants modulate N2 fixation to match N accumulation remains to be determined. A transitory or continued excretion of excess NH4+ produced by highly efficient microsymbionts remains as one possibility.

It is apparent from data in Figure 4 that N2 fixation rates in 26-day-old peas were not sufficient evidence on which to predict whole-plant N content. Nevertheless data from these experiments show that Rhizobium strains which are capable of providing greater quantities of reduced N can affect photosynthetic rates and growth of peas during vegetative growth. Whether such benefits extend beyond flowering (day 28) to influence total biomass productivity and seed yield is unknown.

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