

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

II. HOST PLANT RESPONSE TO NITROGEN FIXATION BY *RHIZOBIUM* STRAINS¹

Received for publication November 11, 1977 and in revised form February 22, 1978

GABOR J. BETHLENFALVAY, SALAH S. ABU-SHAKRA,² AND DONALD A. PHILLIPS
Department of Agronomy and Range Science, University of California, Davis, California 95616

ABSTRACT

Physiological responses to infection by strains of *Rhizobium leguminosarum* which differed in their capacity to reduce N₂ 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 N₂ reduction rates, calculated from H₂ evolution and C₂H₂-dependent C₂H₄ production measurements, were approximately 6-fold greater than the lowest. Higher N₂ fixation rates were associated with greater CO₂ exchange rates (R² = 0.92) and carboxylation efficiency (R² = 0.99). Increases in the apparent relative efficiency of N₂ fixation [1-(H₂ evolved in air/C₂H₂ reduced)] (acteroid efficiency) were associated with increases in whole-plant N₂ fixation efficiency (N₂/CO₂ reduction ratio) (R² = 0.95). Whole-plant dry weight and total N content were related by regression analysis (R² = 0.98); both parameters were enhanced by increased N₂ fixation in a manner analogous to previously reported increases caused by greater external applications of NH₄⁺. These data reveal that photosynthetic parameters in genetically uniform host plants grown under identical environmental conditions are affected by N₂ fixation characteristics of the rhizobial symbiont. The measured efficiencies of micro- and macrosymbiont are directly related under uniform environments.

Dependence of N₂ fixation on photosynthetic products is a concept supported by data of numerous investigations (3, 4, 6, 10). It is axiomatic that the capacity of the photosynthetic apparatus to provide required photosynthate for all plant functions including N₂ fixation is controlled to a large extent by the availability of reduced N. Previous work has shown that photosynthesis in 26-day-old Alaska peas (*Pisum sativum* L.) is strongly influenced by availability of reduced nitrogen, whether it is supplied exogenously or by the *Rhizobium* symbiosis (2). The present study was begun to determine whether photosynthesis in a genetically uniform host legume grown under controlled conditions could be altered by providing *Rhizobium* strains which differed in their capacity to reduce N₂.

MATERIALS AND METHODS

Pea (*P. sativum* L. cv. Alaska) plants were maintained, and

¹ This material is based on research supported by National Science Foundation Grant AER 77-07301. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the National Science Foundation.

² Recipient of a Research Associate Award from the International Development Research Center, Canada. Permanent address: Department of Crop Production and Protection, American University of Beirut, Lebanon.

photosynthesis, N₂ 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, N₂ fixation, RE³ (9), CER, CE, and C₂H₂ 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 N₂ fixation to CER, N₂ fixation to CE, N₂ fixation to N content, N content to dry weight, and RE to N₂/CO₂ 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 [$y = a(1 - be^{-cx})$] for the first three relationships, on the linear function for the fourth, and on the exponential function ($y = ae^{cx}$) for the last relationship.

RESULTS AND DISCUSSION

Symbiotic N₂ fixation rates were calculated from C₂H₂ reduction and H₂ evolution data for all *Rhizobium* strains tested in association with Alaska pea (Table I). A comparison of C₂H₂ reduction and H₂ production with the computed N₂ fixation values obtained in this experiment supports the concept (9) that C₂H₂ reduction values can misrepresent N₂ fixation in the absence of H₂ evolution data from the same root nodules. Of 10 possible comparisons between strains for N₂ fixation and RE, only one (128C53-92A2) was not significantly different ($P \leq 0.05$) in Duncan's multiple range test (5). The same procedure, however, revealed that only strain 92F1 differed significantly from the other four strains in C₂H₂ reduction capacity. One interpretation of these data is that strains 128C53 and 92A2 had a greater rate of calculated N₂ fixation than strains TA101 and 175G10 because the former evolved less H₂ as reflected in the RE values. Strain 92F1 had enhanced N₂ fixation not only because of a greater RE but also because of a greater flux of electrons passed through the nitrogenase complex (greater C₂H₂ reduction). Thus, there may be at least two genetic mechanisms in *Rhizobium* by which N₂ fixation can be enhanced.

Regression analysis of RE and N₂/CO₂ values in Table I revealed that these data fitted a nonlinear function ($y = 0.31 e^{3.63x}$) with R² = 0.95 ($P \leq 0.05$). This finding suggests that under uniform environmental conditions, such as those used in this study, increases in RE of the microsymbiont were associated with

³ Abbreviations: CE: carboxylation efficiency; CER: CO₂ exchange rate; C₂H₂ reduction: C₂H₂-dependent C₂H₄ production; RE: apparent relative efficiency of N₂ fixation [1-(H₂ evolved in air/C₂H₂ reduced)].

Table I. Nitrogen fixation of five *Rhizobium* strains on *Pisum sativum* L. cv. Alaska. Means and standard errors were computed from five replicates. Specific activity of N_2 fixation was calculated as $[(C_2H_2 \text{ reduced} - H_2 \text{ evolved})/3]/\text{g nodule dry wt}\cdot\text{hr}$. The N_2/CO_2 data represent the ratio of μmoles of symbiotic N_2 and photosynthetic CO_2 reduction per plant per hour and were computed from the means of five and three replicates, respectively.

Trait	<i>Rhizobium</i> Strain				
	TA101	175G10	128C53	92A2	92F1
C_2H_2 Reduction ($\mu\text{mole } C_2H_4/\text{plant}\cdot\text{hr}$)	4.55 \pm 0.92	6.30 \pm 0.36	5.02 \pm 0.27	5.14 \pm 0.23	12.26 \pm 1.10
H_2 evolution ($\mu\text{mole } H_2/\text{plant}\cdot\text{hr}$)	3.86 \pm 0.49	5.31 \pm 0.32	2.64 \pm 0.30	2.65 \pm 0.25	5.77 \pm 0.72
N_2 Fixation ($\mu\text{mole } N_2/\text{g nodule dry wt}\cdot\text{hr}$)	4.6 \pm 2.4	7.8 \pm 1.0	13.8 \pm 2.1	11.7 \pm 0.5	29.0 \pm 1.6
RE [1 - (H_2 evolved/ C_2H_2 reduced)]	0.07 \pm 0.02	0.16 \pm 0.02	0.47 \pm 0.04	0.48 \pm 0.03	0.59 \pm 0.03
$N_2/CO_2 \times 10^3$	0.37	0.65	1.46	1.41	3.35

increased macrosymbiont efficiency (N_2/CO_2 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 N_2/CO_2 uptake ratios.

Symbiotic N_2 fixation, when based on both C_2H_2 reduction and H_2 evolution data, was related positively with CER ($R^2 = 0.92$) and CE ($R^2 = 0.99$) as indicated by regression equation $y = a(1 - be^{-cx})$ (Figs. 1 and 2). The fact that CER increased with greater N_2 fixation may be explained by the possibility that more N_2 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 CO_2 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 $y = 16.49x + 0.3109$ ($R^2 = 0.98$) (Fig. 3). The capacity of the microsymbiont to provide NH_4^+ 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 NH_4^+ supply (2). Plants grown with less efficient rhizobial strains had total N contents comparable to nonsymbiotic plants provided with smaller amounts of NH_4^+ . Total plant dry weight, however, was significantly greater ($P \leq .01$) in the nonsymbiotic peas grown with 8 and 16 mM NH_4^+ (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 N_2 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 N_2 -fixing symbiosis had a greater dry weight 26 days after germination. This fact is a consequence of the interdependence of photosynthesis and N_2 fixation. Although both the N_2/CO_2 uptake ratio (Table I) and whole-plant CER (Fig. 1) increased with greater N_2 fixation, it is apparent that neither symbiotic N_2 fixation nor photosynthetic CO_2 reduction could increase without limits. In Figure 1 data fitted to the function $y = 30.57(1 + 0.73e^{-2.02x})$ ($R^2 = 0.92$) suggest that the coefficient

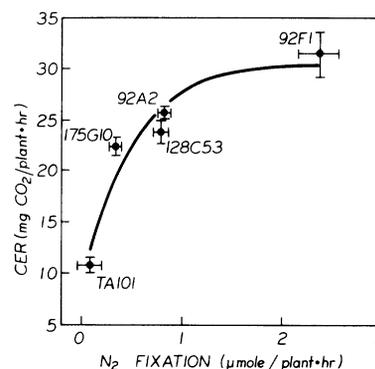


FIG. 1. Relationship between symbiotic N_2 fixation and CO_2 exchange rate (CER) in 26-day-old pea plants inoculated with different strains of *Rhizobium*. Means \pm SE were computed from five and three replicates respectively. Annotation adjacent to data points refers to the strain number of *R. leguminosarum* used as inoculant. Nitrogen fixation was computed as $(C_2H_2 \text{ reduced} - H_2 \text{ evolved})/3$. Data were fitted to a nonlinear function $[y = 30.57(1 + 0.73e^{-2.02x})]$ with $R^2 = 0.92$ ($P \leq 0.05$).

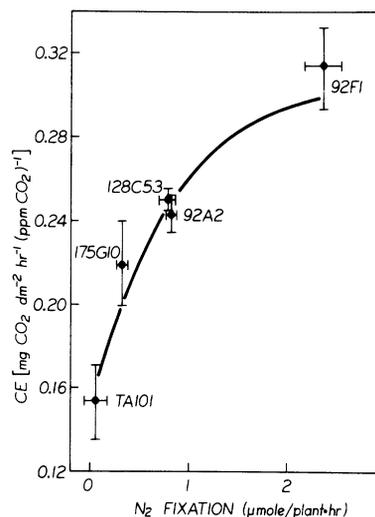


FIG. 2. Relationship between symbiotic N_2 fixation and carboxylation efficiency (CE) in 26-day-old peas inoculated with different strains of *Rhizobium*. Annotation, computations, and plants used were as in Figure 1. Data were fitted to a nonlinear function $[y = 0.32(1 + 0.55e^{-1.27x})]$ with $R^2 = 0.99$ ($P \leq 0.01$).

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 CO_2 exchange under the experimental

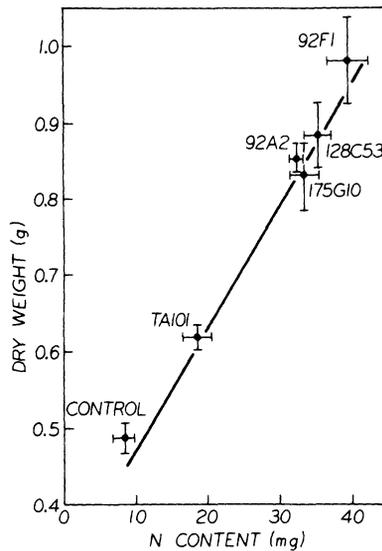


FIG. 3. Relationship between total plant N content and plant dry weight in 26-day-old pea plants inoculated with different strains of *Rhizobium*. Annotation of data points was as in Figure 1 except for control plants which were not inoculated. Means \pm SE were computed from eight replicates. Data were fitted to a linear function ($y = 16.49x + 0.3109$) with $R^2 = 0.98$ ($P \leq 0.01$).

conditions. A similar argument may be applied to the N_2 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 N_2 fixation to match N accumulation remains to be determined. A transitory or continued excretion of excess NH_4^+ produced by highly efficient micro-symbionts remains as one possibility.

It is apparent from data in Figure 4 that N_2 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.

Acknowledgment The authors appreciate advice on statistical analysis provided by W. A. Williams.

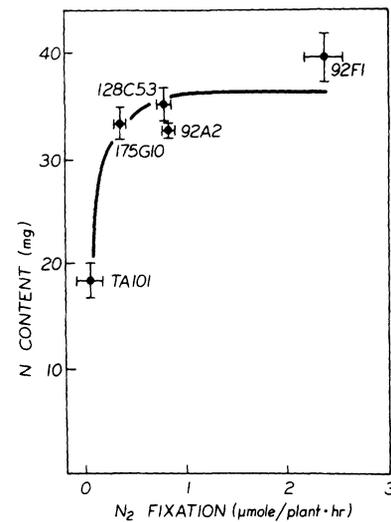


FIG. 4. Relationship between symbiotic N_2 fixation and total N content in 26-day-old pea plants inoculated with different strains of *Rhizobium*. Means \pm SE were computed from eight replicates. Annotation, computation of N_2 fixation, and plants used were as in Figure 1. Data were fitted to a nonlinear function [$y = 35.84 (1 + 1.01 e^{-8.11x})$] with $R^2 = 0.91$ ($P \leq 0.05$).

LITERATURE CITED

- AUGUSTINE JJ, MA STEVENS, RW BREIDENBACH, DF PAIGE 1976 Genotypic variation in carboxylation of tomatoes. *Plant Physiol* 57: 325-333
- BETHLENFALVAY GJ, SS ABU-SHAKRA, DA PHILLIPS 1978 Interdependence of nitrogen nutrition and photosynthesis in *Pisum sativum* L. I. Effect of combined nitrogen on symbiotic nitrogen fixation and photosynthesis. *Plant Physiol* 62: 127-130
- BETHLENFALVAY GJ, DA PHILLIPS 1977 Ontogenetic interactions between photosynthesis and symbiotic nitrogen fixation in legumes. *Plant Physiol* 60: 419-421
- BETHLENFALVAY GJ, DA PHILLIPS 1977 Effect of light intensity on efficiency of carbon dioxide and nitrogen reduction in *Pisum sativum* L. *Plant Physiol* 60: 868-871
- DUNCAN DB 1955 Multiple range and multiple *F* tests. *Biometrics* 11: 1-42
- HARDY RWF, UD HAVELKA 1976 Photosynthate as a major factor limiting nitrogen fixation by field grown legumes with emphasis on soybeans. In PS Nutman, ed, *Symbiotic Nitrogen Fixation in Plants*. Internat Biol Programme, Vol. 7. Cambridge Univ Press, Cambridge, pp 421-439
- OGHOHORIE CGO, JS PATE 1971 The nitrate stress syndrome of the nodulated field pea (*Pisum arvense* L.). In TA Lie, EG Mulder, eds, *Biological Nitrogen Fixation in Natural and Agricultural Habitats*. *Plant Soil* (special volume) pp 185-202
- PATE JS 1975 Pea. In LT Evans, ed, *Crop Physiology*. Cambridge Univ Press, Cambridge, pp 191-224
- SCHUBERT KR, HJ EVANS 1976 Hydrogen evolution: a major factor affecting the efficiency of nitrogen fixation in nodulated symbionts. *Proc Natl Acad Sci USA* 73: 1207-1211
- WILSON PW 1935 The carbohydrate-nitrogen relation in symbiotic nitrogen fixation. *Res Bull* 129, Agric Exp Sta, Univ Wisconsin, Madison, pp 1-40