HORTSCIENCE 21(2):287–289. 1986.

Host Plant Traits Associated with Estimates of Nodulation and Nitrogen Fixation in Common Bean

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Additional index words. acetylene reduction, visual nodule score, *Phaseolus vulgaris*, *Rhizobium phaseoli*

Abstract. **Field and greenhouse experiments were conducted to study the relationships among plant growth traits; ratios of dry weights among nodules, roots, and shoots; and traits associated with N_2-fixation potential of common bean (*Phaseolus vulgaris* L.). The dry weights of plant parts and the traits associated with N_2-fixation differed among the 10 lines studied. A visual nodulation score used to evaluate N_2-fixation potential was correlated positively with nodule dry weight, acetylene reduction (AR) value, root dry weight, and shoot dry weight for plants grown under both greenhouse and field conditions. AR values, nodule dry weight, and visual nodule scores of plants grown in the greenhouse were correlated positively with the respective figures obtained for field-grown plants. These methods of evaluation can be used to discriminate among lines for N_2-fixation potential.**

Genotypic variation for N_2-fixation has been reported in common bean (5, 6, 8, 15), and photosynthate partitioning has been suggested as an important factor influencing N_2-fixation (5, 7, 9). Shoot:root (S:R) and root:nodule (R:Nd) ratios (indicating distribution of dry matter among the shoot, root, and nodules) have been used to express the partitioning of photosynthate available for N_2-fixation. Studies with cowpea (12), white clover (10), soybean (11), and bean (6, 8, 14) have shown that S:R and R:Nd ratios are usually lower in nodulated plants, but that environmental factors including N fertilization also affect partitioning of dry matter and its relationship to N_2-fixation (2, 11, 12).

Breeding for enhanced N_2-fixation is an attractive approach to raising bean yields and reducing fertilizer N requirements in areas where available soil nitrogen is limited and fertilizer is difficult to obtain or expensive (4). The studies reported were conducted under field and greenhouse conditions to determine relationships between N_2-fixation and dry matter content of shoots, roots, and nodules and to test a visual scoring system for evaluating nodulation of bean plants.

The 10 common bean lines used in this study differed in seed color, plant type, and time to 50% bloom and maturity (Table 1). The Univ. of Wisconsin (UW) lines were inbred backcross lines developed from a cross of 'Sanilac' x 'Puebla 152', and selected for differences in N_2-fixation potential based on acetylene reduction (AR) values at the R3 growth stage and for seed yield at maturity (8).

The field experiment was conducted at the Hancock Experiment Station, Hancock, Wis. The soil was a Plainfield loamy sand with a pH of 6.3, with 9 MT of organic matter per hectare, 170 kg P per hectare, and 270 kg K per hectare. To supply adequate P and K, 100 $kg \cdot ha^{-1}$ of 0N–0P–48K were broadcast uniformly and incorporated into the soil prior to planting, and 120 $kg \cdot ha^{-1}$ of 0N–14P–42K were band-applied at planting time.

Seeds were treated with a 50/50 (v/v) mixture of thiram fungicide and *O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate (diazinon) insecticide immediately before planting (2 June 1984). Each treatment consisted of an individual line assigned to a single row 4.5 m long with 30 plants spaced 15 cm apart in rows. The distance between rows was 90 cm. The treatments were distributed in a randomized, complete block design with 4 replicates. Two seeds were dropped in pre-made holes in the rows, and thinned to a full stand 2 weeks after planting. Granular inoculant of *Rhizobium phaseoli* (CIAT 75 and 676, Allen 413-2, and Kimberly 5) was hand-applied into each hole with the seeds in the inoculated plots before covering. The *R. phaseoli* strains, chosen for their proven effectiveness, were obtained from the Nitragin Co. (Milwaukee). Usual recommended cultural practices were followed (8).

A 10-plant sample was taken from each treatment row at 56 days after planting (DAP), corresponding to the R3 stage of development (50% bloom) of 'Porrillo Sintetico', which was used as the control line. The nodulated roots of plants in each sample were assayed for AR using the procedure of Graham and Rosas (6), washed immediately, and scored for nodulation using a visual comparison to the nodule mass of plants of the control line. Nodule, root, and shoot samples were collected, dried (48 hr at 70°C), weighed, and used to calculate S:R and R:Nd ratios (dry weight:dry weight). Seed yield of

Table 1. Characteristics of the common bean lines grown in field (Hancock, Wis., 1982) and greenhouse (Madison, Wis., 1983) studies.

			No. of days		
			50% bloom		Maturity
Line	Seed color	Plant type[z]	Field	Greenhouse	(field)
UW 24-17[y]	white	III	54	55	116
UW 24-21	white	II	52	50	97
UW 24-40	white	II	48	52	97
UW 24-55	white	II	52	53	106
Sanilac	white	I	47	50	90
Puebla 152	black	III	55	60	116
Porr. Sint.	black	II	56	60	113
Jamapa	black	II	51	60	106
Ex Rico 23	white	I	47	50	97

[z]Type I = determinate-bush; Type II = indeterminate-bush, erect branches; and Type III = indeterminate bush, prostrate branches.
[y]UW lines are near-homozygous lines developed by the inbred backcross line method (8).

Table 2. Method to determine visual rating of root systems.

Visual rating	Nodulation class	Subjective description
1	Poor nodulation	< 50% nodulation of control
2	Below average	51–80% of control
3	Average level	81–120% of control
4	Higher than average	121–150% of control
5	Highly superior	>150% of control

Received for publication 4 Mar. 1985. Research supported by funds provided by The College of Agricultural and Life Sciences and the Graduate School, Univ. of Wisconsin–Madison, and the AID–S&E/CSRS Program, under grants nos. 801-15-36 and 59-2551-1-5-006-0. This research is part of a thesis submitted by J.C.R. in partial fulfillment of the requirements for the PhD. We gratefully acknowledge the assistance of Ken Kmiecik in conducting this research. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the Univ. of Wisconsin–Madison and does not imply its approval to the exclusion of other products or vendors that may be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.
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Table 3. Variation in traits related to plant growth and N_2-fixation measured at the R3 growth stage of common bean plants inoculated with *Rhizobium phaseoli* and grown in the field (Hancock, Wis., 1982) and the greenhouse (GH) (Madison, Wis., 1983).

	Nodule dry wt[z]		AR value[y]		Nodule score[x]		Root dry wt[w]		Shoot dry wt[w]		Total dry wt[y]		Root:nodule[v]		Shoot:root[v]	
Lines	Field	GH	Field	GH	Field	GH	Field	GH	Field	GH	Field	GH	Field	GH	Field	GH
Puebla 152	220	473	9.7	46.6	3.0	3.8	0.9	1.1	10.5	8.8	11.6	10.3	3.9	2.3	13.3	8.1
Porrillo Sint.	148	400	11.5	41.2	3.0	3.0	1.2	1.4	10.1	8.8	11.4	10.5	8.6	3.5	8.6	6.1
UW 24-17	107	287	6.5	40.3	2.2	2.8	1.0	1.1	9.1	8.1	10.2	9.5	9.7	3.8	9.8	10.9
UW 24-21	90	165	4.1	3.9	2.0	2.5	1.3	1.0	9.1	10.8	10.5	11.9	15.2	6.1	7.0	10.8
UW 24-55	84	205	5.8	10.9	2.4	2.0	0.9	1.1	5.9	7.5	6.9	8.8	13.6	5.6	7.4	6.9
UW 24-40	71	275	2.8	20.0	1.9	3.0	0.9	1.2	6.6	10.6	7.6	12.1	13.6	4.5	7.4	8.8
UW 24-63	65	218	1.4	13.2	1.8	2.0	0.8	0.8	6.0	7.4	6.8	8.5	12.4	3.9	7.9	9.1
Jamapa	62	257	3.7	12.5	1.4	2.3	0.9	0.9	8.8	4.5	9.7	5.7	13.9	3.8	11.3	5.1
Ex Rico 23	53	173	2.8	9.6	1.8	2.3	1.0	0.7	7.0	11.1	8.0	12.1	19.5	4.2	7.7	16.5
Sanilac	41	116	1.3	2.1	1.6	1.3	0.8	0.8	6.6	6.4	7.5	7.3	20.4	6.9	8.4	8.3
Mean	98	257	6.1	20.0	2.2	2.5	1.0	1.0	8.0	8.4	9.0	9.7	12.3	4.5	8.8	9.1
LSD 5%	43	68	6.1	13.1	0.6	0.7	0.4	0.2	NS	3.1	NS	0.5	6.8	1.1	NS	3.2

[z]mg per plant.
[y]Acetylene reduction (AR) values (μmol $C_2H_2 \cdot plant^{-1} \cdot hr^{-1}$).
[x]1 = poor nodulation, 2 = below average, 3 = average level, 4 = higher than average, and 5 = highly superior.
[w]Gram per plant.
[v]Dry-weight ratios.
NS = Nonsignificant.

one 10-plant sample per row was determined at maturity.

The same bean lines were grown in an experiment conducted in the greenhouse. Natural light conditions were supplemented with a 12-hr illumination period throughout the experiment using fluorescent lamps (cool-white, 160W). Average temperature in the greenhouse was about 22° ± 2°C. Each experimental unit was one pot containing one plant and was arranged in a randomized complete block design with 4 replications. The experiment was begun 11 Jan. 1983. Seeds were surface-sterilized with 10% Clorox (H_2O_2) bleach for 2 min and were germinated for 3 days, planted, and inoculated with the *R. phaseoli* granular inoculum as described for the field experiments. Three seeds were sown in each 15-cm pot containing a sand:perlite (1:1) medium. Plants were thinned to one per pot one week after emergence and watered twice a week using the nutrient solution of Summerfield et al. (13) containing 20 mg of N per liter; in addition, tap water was used to maintain optimum moisture conditions. Plants were harvested at 60 DAP, which corresponded to the R3 stage of development of 'Porrillo Sintetico', the control line.

A composite nodulation score (NS) was determined based on visual rating of the nodulation (nodule number and mass) of each root system in the experimental lines compared to roots of the control line. For each experimental sample, NS = Σ (rating x n)/N = composite nodulation score, where n = number of roots with each rating and N = total number of roots per sample. Ratings of 1 to 5 were determined by the criteria shown in Table 2.

Significant differences (P = 0.05) were detected among lines for nodule dry weight, AR value, nodule score, root dry weight, and R:Nd in the field and greenhouse experiments (Table 3). Significant differences among lines were detected only in the greenhouse for shoot and total plant dry weight and S:R. Nodule score was correlated positively with AR value and nodule dry weight in both field and greenhouse experiments, suggesting that each of these measurements could be used to identify lines with superior N_2-fixation potential (Table 4). Nodule dry weight and nodule score were correlated positively with root dry weight and were correlated negatively with R:Nd in both experiments. These results suggest that high N_2-fixation potential in these bean lines was associated with large root systems that allowed development of and supported a large nodule mass. Some patterns of correlations between other plant traits were not consistent between the field and greenhouse experiments.

The use of a visual score to evaluate nodulation and N_2-fixation potential between lines has been suggested previously (1, 3). Herein, the nodulation scores were correlated positively to nodule dry weight and AR values

Table 4. Correlation coefficients (r) between traits related to plant growth and N_2-fixation measured at the R3 growth stage of common bean plants inoculated with *Rhizobium phaseoli* and grown in the field (Hancock, Wis., 1982) and the greenhouse (Madison, Wis., 1983)[z].

		Trait						
Trait		Root dry wt	Shoot dry wt	Total dry wt	Shoot: root	Root: nodule	Nodule dry wt	AR value[y]
Shoot dry wt	Field	0.42**						
	Greenhouse	NS						
Total dry wt	Field	0.51**	0.99**					
	Greenhouse	NS	0.99**					
Shoot:root	Field	0.50**	0.52**	0.44**				
	Greenhouse	−0.52**	0.66**	0.60**				
Root:nodule	Field	NS	NS	NS	−0.44**			
	Greenhouse	NS	NS	NS	NS			
Nodule dry wt	Field	0.38**	0.51**	0.54**	NS	−0.66**		
	Greenhouse	0.55**	NS	NS	NS	−0.77**		
AR value	Field	NS	0.36**	0.37**	NS	−0.55**	0.65**	
	Greenhouse	0.47**	NS	NS	NS	−0.70**	0.90	
Nodule score	Field	0.48**	0.46**	0.50**	NS	−0.46**	0.80**	0.58**
	Greenhouse	0.49**	0.32*	0.36	NS	−0.64**	0.79**	0.64**

[z]Samples were taken at the R3 stage of development of 'Porrillo Sintetico'.
[y]Acetylene reduction (AR) values (μmol $C_2H_2 \cdot plant^{-1} \cdot hr^{-1}$).
*,**,NS = significant at $P \leq 0.05$, $P \leq 0.01$, and nonsignificant, respectively.

Table 5. Correlation coefficients (r) between values recorded for field and greenhouse-grown plants for traits related to plant growth and N_2-fixation measured at the R3 growth stage of common bean plants inoculated with *Rhizobium phaseoli*.[z]

Traits	Correlation coefficients (r)
Root dry wt	0.32*
Shoot dry wt	NS
Total dry wt	NS
Shoot:root	NS
Root:nodule	0.43**
Nodule dry wt	0.72**
Acetylene reduction	0.62**
Nodule score	0.48**

[z]Samples were taken at the R3 stage of development of 'Porrillo Sintetico'.
*,** NS = significant at $P \leq 0.05$, $P \leq 0.01$, and nonsigificant, respectively.

in both the field and greenhouse experiments (Table 4).

The visual scoring system should be useful where many lines are to be evaluated, with the more promising lines being evaluated further with more precise techniques (e.g., AR assay and use of ^{15}N-labeled fertilizer). For breeding programs, visual nodulation scores in addition to seed yields of nodulated plants grown under low soil N conditions can provide a means of estimating N_2-fixation potential of breeding lines.

Among the traits measured, significant positive correlations between field and greenhouse values were obtained for root dry weight, R:Nd, nodule dry weight, AR value, and nodule score (Table 5). These positive values suggest that greenhouse screening can be used to eliminate lines having poor N_2-fixation potential, but for more precise evaluation of field potential, the remaining lines should be tested in the field using appropriate techniques.

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HortScience 21(2):289–291. 1986.

Growth of Ovaries of Parthenocarpic and Nonparthenocarpic Tomato Genotypes in Vitro

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Additional index words. *Lycopersicon esculentum* Mill.

Abstract. **Newly opened flowers of 4 parthenocarpic tomato (*Lycopersicon esculentum* Mill.) genotypes, PSET-1 (*pat-2*), 'Severianin (*pat-2*), RP 75/59, and Stock 2524 (*pat*), and 3 nonparthenocarpic genotypes, 'Walter', 'Flora-Dade', and 'Homestead 24', were excised and placed on nutrient media. The ovaries of the parthenocarpic genotypes increased in diameter and weight to a much greater extent than the ovaries of the nonparthenocarpic genotypes during a 6-day period at 25°C. In other comparisons, the parthenocarpic genotypes had larger ovaries than the nonparthenocarpic genotypes after 2, 4, 6, 12, 18, and 24 days. The ovaries of buds excised 1 and 3 days before anthesis and at anthesis from PSET-1 and 'Severianin' were larger after 6 days than those excised from 'Walter' and 'Flora-Dade'.**

Nitsch (5) found that excised tomato ovaries cultivated in vitro retained the general growth pattern of fruits grown on the plant, although the final size was much smaller. Asahira and Hosoki (1) induced parthenocarpy in excised tomato ovaries before making in vitro studies on puffiness.

Mapelli et al. (3) found that ovaries developing on a parthenocarpic tomato line containing the *pat* gene increased in weight faster during the first week following anthesis than ovaries on an isogenic nonparthenocarpic cultivar. Seeded fruit attained their highest growth rate between the 15th and 20th day after anthesis.

Received for publication 5 Apr. 1985. Florida Agricultural Experiment Stations Journal Series No. 6333. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

The present research was undertaken to determine if such differential growth would occur with parthenocarpic and nonparthenocarpic genotypes when flowers were excised and incubated in vitro. Four experiments were conducted to determine ovary growth of opened flowers with time and the effect of bud age on ovary growth.

Expt. 1. The growth of ovaries of flowers of parthenocarpic genotypes PSET-1 (*pat-2*), 'Severianin' (*pat-2*), RP 75/59, and Stock 2524 (*pat*) (2, 6) and of nonparthenocarpic genotypes 'Walter', 'Flora-Dade', and 'Homestead 24' during 6 days on nutrient media was compared. Fifteen flowers of each genotype were used for each determination.

Expt. 2. The size of ovaries of flowers of PSET-1 and 'Homestead 24' genotypes was determined after 2, 4, and 6 days on nutrient media. Twenty flowers were used for each determination.

Expt. 3. The size of ovaries of flowers of PSET-1, 'Severianin', 'Walter', and 'Flora-