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Symbiotic $N_2(C_2H_2)$ Fixation by Bean[1]

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ABSTRACT

The response of bean (*Phaseolus vulgaris* L.) to N fertilization under field conditions indicates different *Rhizobia*-cultivar relationships or symbiotic N_2-fixation limitations due to the cultivars. We have used the acetylene reduction method to determine the relative seasonal $N_2(C_2H_2)$ fixation of several field-grown bean cultivars. The relative nitrogenase activity was estimated from 8- × 15-cm soil cores taken around the main root of a decapitated plant. Activities rapidly increased from the three-node vegetative (V3) to early pod-filling (R3-R4) growth stages, thereafter decreasing to zero at physiological maturity (R9). Accumulated daily activity totals showed a five- to sixfold difference in seasonal $N_2(C_2H_2)$ fixation between cultivars, which was significantly related to the average nodule weight and to the plant dry weight near physiological maturity. However, cultivars with similar plant dry weights had a two- to threefold difference in relative $N_2(C_2H_2)$ fixation. Seed yields and total-N uptakes were also positively related. These observations indicate that it may be possible to increase both the symbiotic N_2 fixation and seed yields through plant breeding.

Additional index words: **Acetylene reduction assay, N uptake, Seed yields, Nodulation *Phaseolus vulgaris***

WORLDWIDE interest in biological N_2-fixing systems has been brought about by uncertain availability and higher cost of N fertilizers, as well as recently developed analytical tools that critically evaluate biological N_2-fixation activities.

Different utilization-efficiencies of K (19), P (9, 23), and Zn (3) have been shown for some bean cultivars. The response of this species to N fertilization under some field conditions is also dependent upon the cultivar (4, 9), indicating that there may be a range of effectiveness for the *Rhizobia*-cultivar relationship or a N_2-fixation limitation due to the characteristics of the cultivar itself. Different $N_2(C_2H_2)$ fixation profiles for soybean (*Glycine max* (L.) Merr.) cultivars (12, 17) have resulted in significantly different seasonal N_2-fixation totals. Relationships between nodulation and seed yields have also been reported (8, 18).

Dry beans and garden beans are generally grown for seed production in southern Idaho without N fertilization and are normally well nodulated from indigenous soil *Rhizobium* populations. Southern Idaho is also one of the major seed-producing areas in the United States, with numerous *Phaseolus* cultivars produced for seed each year. We undertook a study to evaluate the symbiotic-nonsymbiotic N relationships of bean grown for seed. We report here a relative measure of the seasonal nitrogenase activities of several cultivars using the acetylene reduction (AR) assay (12).

MATERIALS AND METHODS

Eighteen bean cultivars (Table 1) were grown on a Portneuf silt loam soil at Kimberly, Idaho. The Portneuf soil (Xerollic calciorthid) has a calcic horizon beginning at about 45 cm, which restricts root growth but not water movement. The beans were seeded on 27 May 1975 in 61-cm rows to give plant populations of about 87,000 and 120,000 plants/ha for determinate and indeterminate plant types, respectively. The actual plant population for each cultivar was recorded on four randomly selected 1.5-m row sections at mid-bloom, R3 growth stage (16) (a system similar to that describing soybean growth (6)). The beans were furrow-irrigated in furrows (122 cm apart) when tensiometers at a 20- to 25-cm depth in the row indicated that about 55% of the available soil moisture remained. Seed yields were determined from three replications of two rows, each 18 m long.

The AR assay was used to measure the relative nitrogenase activity seven times during the growing season (1, 11, 12). An 8- × 15-cm soil core was taken between 0900 and 1100 hours from around the tap root of a plant excised at the cotyledonary node. Four plants from each cultivar were selected for uniform top growth and plant spacing. The soil-root cores for each cultivar were combined in a cloth bag and placed in a round PVC tank (26-cm diameter × 30-cm high), closed at one end, and equipped with a detachable lid. The tank was partially evacuated to 0.9 bar and refilled to ambient atmospheric pressure with commercial C_2H_2 that had been scrubbed through concentrated H_2SO_4 and H_2O traps. This system was then incubated for 1 hour at about the same temperautre as that of the soil at the time of sampling. During the incubation period, the gaseous atmosphere inside the tank was continually mixed with an internal fan driven by a brushless external motor. After incubation, duplicate gas samples were placed into 10-ml vacutainers that had been previously evacuated with a vacuum pump and dipped in hot wax.

The C_2H_4 content of the gas sample was determined using a gas chromatograph equipped with dual hydrogen-flame ionization detectors held at 200 C. Nitrogen (N_2) was used as the carrier gas at a flow rate of 25 cm³/min through 1.83- × 0.003-m stainless steel columns packed with 80- to 100-mesh Poropak N. Column and injection port temperatures were maintained at 55 and 100 C, respectively. The ethylene (1-ml sample from each vacutainer) peak areas were proportional to the concentrations over the range utilized by the assays. The retention times for C_2H_4 and C_2H_2 were about 33 and 65 sec., respectively. Ethylene peak areas from duplicate vial-gas samples differed by less than 5%.

After incubation, the soil was washed from the roots and the nodules excised, counted, and their fresh weights determined after equilibration in a 100% relative humidity chamber for 1 hour. The washed roots and the excised plant tops were oven-dried at 60 C, weighed, ground to pass a 40-mesh screen, and analyzed for total N by the semi-micro-Kjeldahl procedure, modified to include nitrates (2).

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Table 1. Characteristics of bean cultivars, 1975.

Cultivar	Seed type	Plant growth type†	Relative maturity‡	Seed yields	Relative $N_2(C_2H_2)$ fixed
			days	q/ha	mg N/plant
UI-114	Pinto	V	96	38.9	55.8
Ouray	Pinto	B	91	35.0	56.7
UI-61	Great Northern	SV	95	36.8	86.9
Idaho Marrow	Large white	SV-B	97	29.2	50.7
Aurora	Small white	SV-B	100	31.3	89.0
Bonus	Small white	SV-B	97	38.0	97.1
Sanilac	Small white	SV-B	96	32.1	68.2
6R396	Small white	SV	97	33.8	58.0
UI-86	Red Mexican	SV	96	37.4	62.3
Mecosta	Kidney	B	103	26.4	55.6
Viva	Pink	SV	90	40.1	91.9
UI-60	Cranberry	B	98	27.9	24.7
SVC	Black turtle	SV	104	28.8	44.6
R275	Black turtle	V	115	33.6	120.1
Swedish Brown	Brown	B	98	31.2	58.6
Canyon	Garden-white	B	110	23.9	23.4
Bush Blue Lake (BBL-274)	Garden-white	B	110	28.8	57.7
Slimgreen	Garden-white	B	110	31.0	47.9

† B = bush; SV = semi-vining; V = vining. ‡ Under south Idaho growing conditions (15).

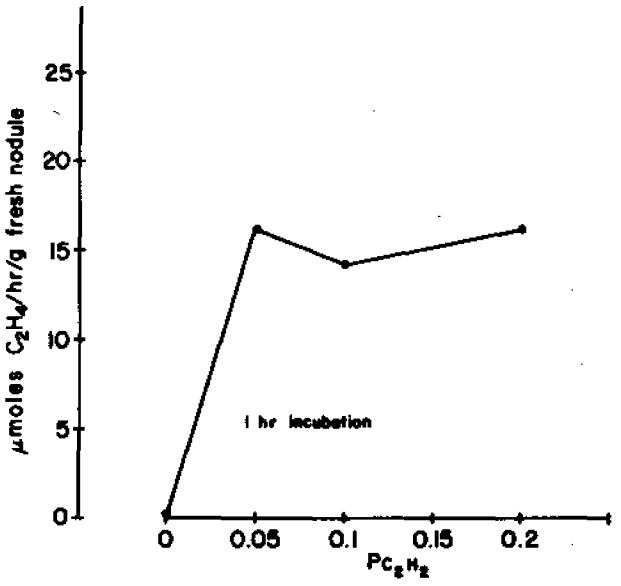

Fig. 1. Ethylene formation from acetylene as a function of Pc_2H_2 by nodulated bean soil-root cores.

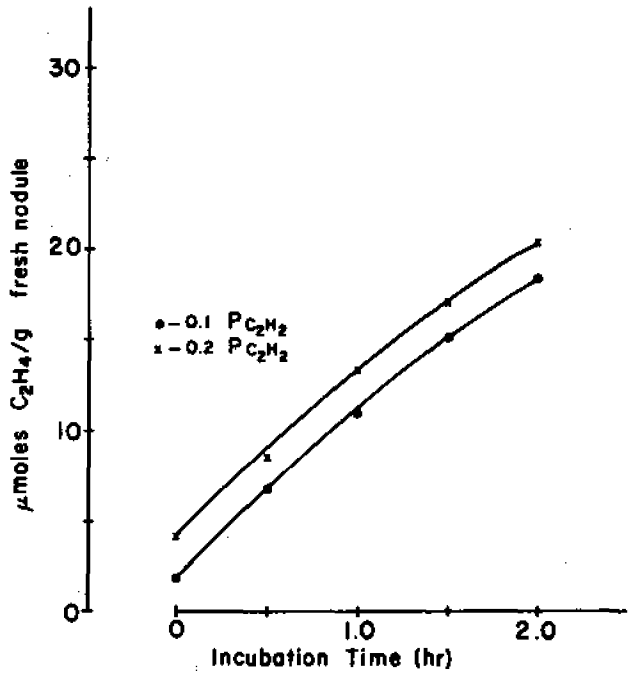

Fig. 2. Time course of acetylene reduction by nodulated bean soil-root cores at two Pc_2H_2. Differences in C_2H_4 between the two curves and that initially measured in the 0.1 Pc_2H_2 is attributed to the C_2H_4 contaminant in the C_2H_2.

RESULTS AND DISCUSSION

Comments on AR Procedure

Preliminary studies indicated that the PVC containers produced no detectable amounts of C_2H_2 or C_2H_4 during the incubation period. In addition, the soil cores with roots and nodules generally produced no detectable amounts of C_2H_4 incubated without C_2H_2. The scrubbed C_2H_2 generally contained a small amount of C_2H_4 contaminant which was determined and subtracted from the post-incubation C_2H_4 concentration.

We found the nitrogenase activity (AR) leveled off at about 0.05 Pc_2H_2 (partial pressure), using the described soil-root core-tank system (Fig. 1). Inhibiting effects were not observed up to 0.2 Pc_2H_2, a level that has been observed to be inhibitory for soybeans (7). In addition, we made no attempt to replace the N_2 in the tanks with a non-substrate inert gas, which may reduce the AR values by 10 to 20% (11, 12). We have used 0.1 Pc_2H_2 for the standard assay.

We followed the kinetics of AR assay in a timed incubation study (Fig. 2). Ethylene concentrations increased without an initial lag period up to the end of a 2-hour incubation period. Lines for the two Pc_2H_2 were also parallel. The initial C_2H_4 concentrations resulted from that amount contained in the C_2H_2, plus that produced in the short reaction time after adding the C_2H_2 but before a gas sample could be withdrawn. Our standard assay samples were taken after 1-hour incubation.

We developed seasonal nitrogenase activity (AR) profiles for each cultivar and determined the area

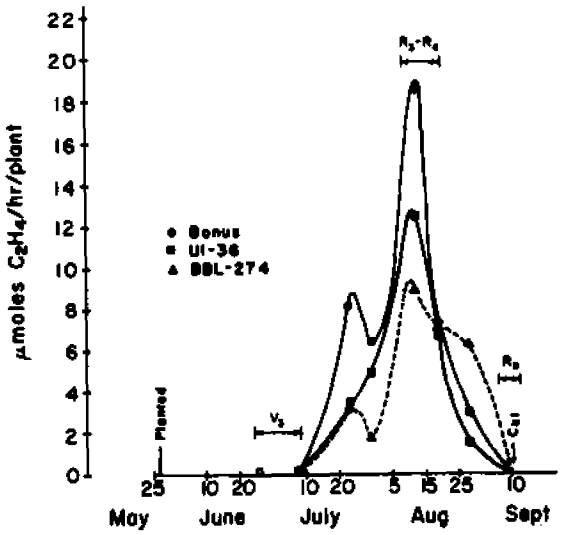

Fig. 3. Seasonal profiles of nitrogenase activity (AR) for three bean cultivars.

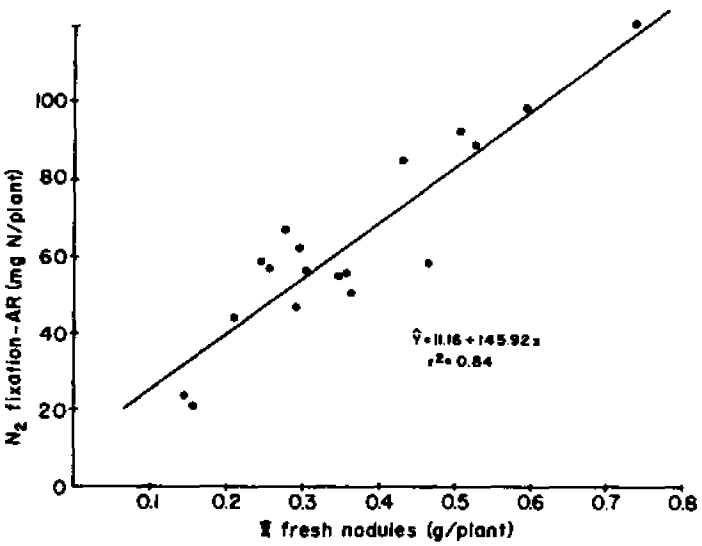

Fig. 4. Relationship between relative $N_2(C_2H_2)$ fixation and seasonal average fresh weight nodules for individual cultivars; significant at 0.01 probability level.

under each profile to estimate the relative activity for the season. A theoretical ratio of 3:1 moles C_2H_4: moles N_2 (11, 12) was used to convert µmoles C_2H_4 to mg N/plant for comparison of the relative $N_2(C_2H_2)$ fixation for each cultivar (Table 1). We have also assumed that our AR activity is a mean of that occurring during a 24-hour period. Preliminary measurements showed that this would tend to overestimate the activities for the 24-hour period, but relative comparisons between cultivars would be valid if the cultivars had similar diurnal profiles. The diurnal effects have been related to changes in temperature and photosynthate supply to the nodules (1, 10, 11, 12, 22).

AR of Individual Cultivars

Relative seasonal AR profiles for three selected cultivars are illustrated in Fig. 3. Generally, AR's were small at the first two samplings or until the V3 growth stage (three nodes on main stem). Thereafter, they increased rapidly, peaking at early pod-filling (R3-R4, first bloom pods 3 to 6 cm long) and then decreasing to zero at physiological maturity (R9). The AR for BBL-274 ('Bush Blue Lake 274') did not decrease as rapidly afer peaking as did that of the other two cultivars. This delay of nodule senescence and AR may have been due, in part, to BBL-274's longer growing season as compared with that of 'UI-36' and 'Bonus' (Table 1). Our reported maximum AR's were about two to three times higher than those reported for a dark red kidney cultivar using similar root sampling techniques (14).

The decrease in some profiles at the fourth sampling (1 August) coincided with a general cooling period. Soil temperatures at the 15-cm depth in the plant rows were 20, 15, and 17 C at the third, fourth, and fifth sampling dates, respectively. These data indicated that there may be a cultivar by temperature interaction that affects N_2 fixation.

Maximum AR rates, seasonal profile shapes, and length of the AR period all contribute to the differences in N_2 fixation between cultivars (11). The area under the AR profile for each cultivar has been converted to mg N/plant (Table 1). These totals ranged from a low of 23 to a high of 120 mg N/plant, and are relative since they do not include the entire root system. Visual observations of cross-sectional root profiles on 4 August indicated no readily apparent root and nodule distribution differences between cultivars. The soil-root core was also found to contain approximately 25% of the total AR activity in a 15- × 122-cm cross-section of the root profile.

Two major characteristics are necessary for high levels of symbiotic N_2 fixation: (a) the *Rhizobia* strain(s) in association with the plant must be able to fix large amounts of N_2 and (b) the plant must be able to supply the needed photosynthetic energy and to utilize the fixed N. The AR per nodule weight and the relationship of plant dry weight to symbiotic N_2-fixation activities for the bean cultivars were considered to help evaluate these factors.

The AR was linearly related to the fresh weight of nodules in the soil-root cores when we compared all sampling dates within each cultivar. Slopes of individual cultivar regression lines were between 8.8 and 13.1 µmoles C_2H_4/hour/g fresh weight of nodules, with correlation coefficients (r^2) from 0.51 to 0.96. The slopes of the regression lines for the individual cultivars were not different at the 0.001 probability level. The combined regression equation, which includes all cultivars and sampling dates, is µmoles C_2H_4/hour $= 0.35 + 10.22 \times$ (g fresh nodules), with a correlation coefficient (r^2) of 0.75. The slope (nodule AR activity) resembles that reported for soybeans (7). In summary, the relative $N_2(C_2H_2)$ fixation increased as the average seasonal nodule weight per plant for each cultivar increased (Fig. 4).

Since the data indicated that the AR per nodule mass were similar, the differences in N_2 fixation between *Phaseolus* cultivars were due to different nodule weights per plant. It may also indicate that the *Rhizobium* strain × cultivar interaction was not a signifi-

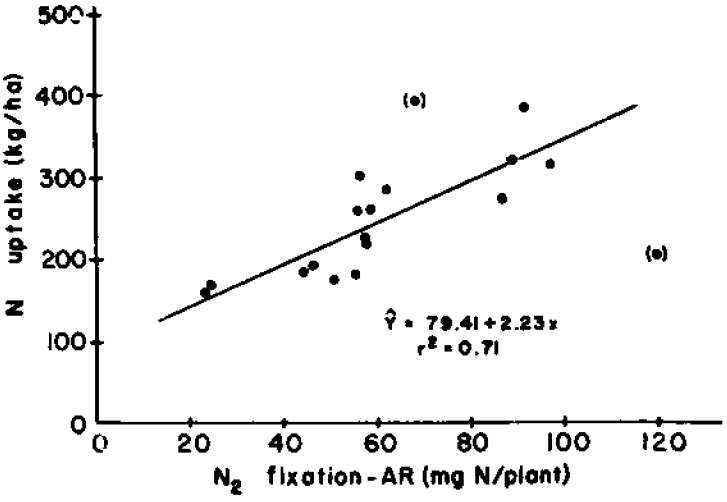

Fig. 5. Relationship between relative $N_2(C_2H_2)$ fixation and total-N uptake for individual cultivars (28 Aug.); significant at 0.01 probability level. ( ) points not included in regression equation.

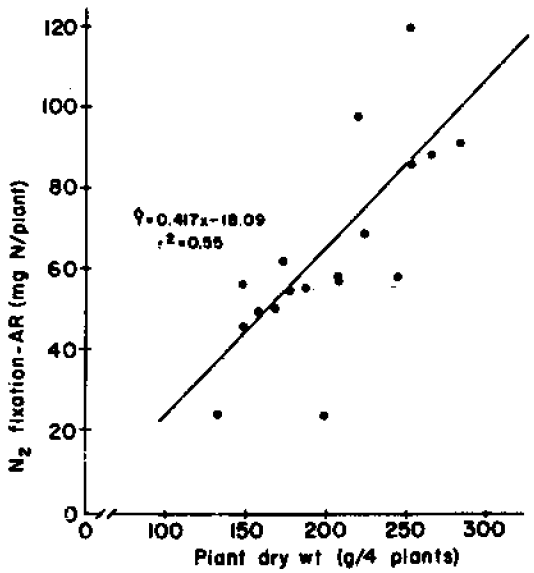

Fig. 6. Relationship between plant dry weights (28 Aug.) and relative $N_2(C_2H_2)$ fixation for individual cultivars; significant at 0.01 probability level.

cant factor in this study, since nodules from different cultivars had similar specific activities. Possibly different *Rhizobia* strains in symbiosis with the lower N_2-fixing cultivars may fix N_2 more effectively than the strains present in this soil. We found no relationships between the AR and nodule numbers per plant or average fresh weight per nodule for any of the cultivars studied. There was a tendency to have more nodules per plant with increased fresh nodule weight per plant, but individual relationships were from different populations.

The relative $N_2(C_2H_2)$ fixation was significantly related to the total-N uptake per area for the cultivars (Fig. 5). We did not include points for the Sanilac and R275 cultivars in the regression equation (Fig. 5) because errors occurred in their total-N uptake determination. Good relationships between the AR assay and N uptake have also been reported for intact rangeland species (22), soybeans (1), white clover (*Trifolium repens* L.) (10, 20), and pasture turfs (21). The bean cultivars (Fig. 5) also tended to be separated into relatively high, medium, and low AR activity groups. This separation indicates that it may be possible to develop cultivars with high N_2-fixation abilities through plant-breeding techniques. About 100 kg N/ha were available to the beans from nonsymbiotic sources (initial residual NO_3-N and organic N mineralized) during the growing season. If all of that were taken up by the bean crop, then from 50 to 250 kg N/ha would be fixed symbiotically.

The plant dry weight near physiological maturity (28 August) was generally related to the relative $N_2(C_2H_2)$ fixation (Fig. 6). This may have been anticipated, since plant dry weights are an integration of all the environmental and genetic factors influencing growth. Significantly, plants with similar dry weights had different $N_2(C_2H_2)$ fixation levels. For example, AR values of 23, 58, 59, 68, and 97 mg N/plant all correspond to approximately 200 g/four plants. This indicated that AR is not specifically related to plant size and that cultivars may have different photosynthetic efficiencies or partitioning to the vegetative, reproductive, or N_2-fixation systems. Differences in photosynthetic rates have been observed among soybeans (5) and bean cultivars (13), but seed yields are, generally, poorly correlated with physiological characteristics. Correlations at earlier sampling dates did not fit as well as those from the 28 August sampling date.

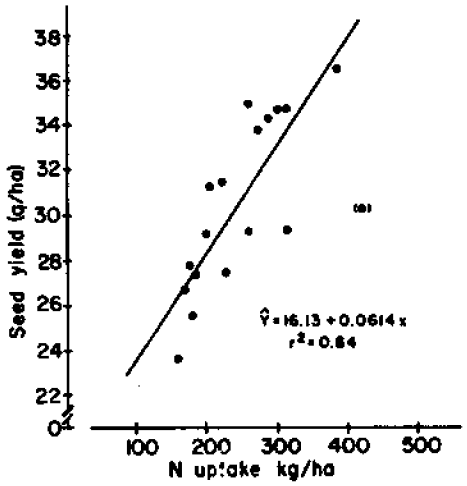

Fig. 7. Relationship between total-N uptake and seed yields; significant at 0.01 probability level. ( ) point not included in regression equation.

The total-N uptakes were significantly related to the seed yields of the cultivars (Fig. 7). In addition, cultivars with high seed yields also had relatively high $N_2(C_2H_2)$ fixations (Table 1, significant at 0.05 probability level). This indicates that it may be possible to develop bean cultivars with both high symbiotic N_2-fixation abilities and seed yields.

SUMMARY

The nitrogenase activity (AR) profiles for bean were found to be similar to those reported for other legumes, starting at early vegetative growth, peaking at early pod filling, and then decreasing to physiological maturity. Differences in cultivars were expressed by the combination of profile shape, maximum AR's, and length of AR activity.

We observed a five- to sixfold difference in relative $N_2(C_2H_2)$ fixation between cultivars, which was related to the average seasonal nodule weight of the individual cultivars and to the plant dry weight near physiological maturity. However, cultivars with similar plant dry weights had a two- to threefold difference in their relative $N_2(C_2H_2)$ fixation. Seed yields and total-N uptake were also related. These observations indicated that it may be possible to increase both the symbiotic N_2 fixation and seed yields of beans by isolating and recombining lines with high N_2 fixation capabilities, as well as improving the *Rhizobium* strain-host symbiosis.

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