

# Correlations between Carbon Isotope Discrimination and Leaf Conductance to Water Vapor in Common Beans<sup>1</sup>

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## ABSTRACT

Carbon isotope discrimination ( $\Delta$ ) was measured in the field on 10 cultivars of common bean (*Phaseolus vulgaris* L.). There was substantial variation (more than 2‰) in leaf  $\Delta$  values and these differences were maintained between vegetative and reproductive developmental stages. These bean lines also exhibited substantial differences in leaf conductance to water vapor, and again these differences were maintained across developmental stages. The differences in leaf conductance were positively correlated with  $\Delta$  values, whether conductance was measured as total leaf conductance or as the individual conductances of either upper or lower leaf surfaces. The observed differences in leaf conductance were not associated with differences in stomatal density. There were small differences among bean lines in their leaf Kjeldahl nitrogen contents, which is interpreted as indicating that photosynthetic capacity among bean lines was similar. Thus, because  $\Delta$  values and leaf conductance were positively correlated, these data suggested that there may have been differences among bean lines in the extent to which stomata limited photosynthetic gas exchange rates.

Leaves of C<sub>3</sub> photosynthetic pathway plants can exhibit substantial variation in carbon isotope composition (<sup>13</sup>C/<sup>12</sup>C), whether it is measured as carbon isotope ratio ( $\delta^{13}\text{C}$ , hereafter referred to as  $\delta_p$ )<sup>2</sup> or as  $\Delta$  (12, 20, 22). There has been increasing interest in the carbon isotopic composition of C<sub>3</sub> plants since it was first shown that  $\delta_p$  was correlated with the ratio of leaf intercellular to ambient CO<sub>2</sub> concentrations ( $c_i/c_a$ ), a parameter which in effect reflects the balance between consumption and supply rates of CO<sub>2</sub> by photosynthetic activities and stomatal diffusion, respectively. Farquhar et al. (13) proposed that  $\delta^{13}\text{C}$  was related to the long-term average  $c_i/c_a$  in C<sub>3</sub> leaves, and shortly thereafter provided the initial supporting evidence for their model (11). To distinguish between the biotic and source factors affecting carbon isotope composition of plant tissues, Farquhar and Richards (14) proposed that  $\Delta$  instead of composition be measured to focus

on the biological processes. From their equations,  $\Delta$  is described as

$$\Delta = a + (b - a)(c_i/c_a) \quad (1)$$

where  $a$  is the isotopic discrimination due to the slower diffusion of <sup>13</sup>CO<sub>2</sub> to <sup>12</sup>CO<sub>2</sub> in air (4.4‰), and  $b$  is the net isotopic discrimination by carboxylation activities (~27‰) (12). The expected correlation between  $\Delta$  and  $c_i/c_a$  has been tested for various time scales: instantaneous (online gas exchange), recently produced sugars and starch which turnover on a scale of days, and whole leaves which turnover on a scale of weeks to months (1, 2, 5, 7, 10, 11, 24). In the crops examined to date (barley, cotton, peanuts, tomato, and wheat), intraspecific variation of up to 3‰ has been observed in carbon isotope discrimination (12).

Common bean (*Phaseolus vulgaris* L.) appears to exhibit at least as much isotopic variation as has been observed for other crop species. In a survey of 99 cultivars, Ehleringer et al. (8) reported that  $\Delta$  values in common bean under rainfed field conditions ranged 3.2‰, and that  $\Delta$  was negatively correlated with maturity date of the crop. In a second study, White et al. (26) reported a 1.7‰ range in  $\Delta$  values, and, furthermore, observed that  $\Delta$  was positively correlated with both yield (dry matter production and seed) and root growth under rainfed conditions.

If  $c_i$  represents the consequence of CO<sub>2</sub> 'supply' and 'demand' rates in photosynthetic gas exchange (15), then changes in  $c_i/c_a$  and therefore in  $\Delta$  could arise as a consequence of variation in  $g$ . That is, a change in the diffusion rate of CO<sub>2</sub> across stomata should result in changes in  $c_i$ . If this were the case, then it is likely that other photosynthetic gas exchange parameters, such as  $g$ , would also be linearly and positively correlated with  $\Delta$ , and that these factors may be contributing to the observed correlations between yield and  $\Delta$ . The purpose of this study was to examine variation in  $\Delta$  among common bean cultivars and to examine correlations between leaf conductance and  $\Delta$ , with the hypothesis that  $g$  and  $\Delta$  should be positively correlated with each other.

## MATERIALS AND METHODS

Ten productive cultivars of common dry bean (*Phaseolus vulgaris* L.) were chosen for these investigations. These were Cahone (pinto type), San Juan Select (pinto type), Sanilac (navy type), Ouray (pinto type), Bonus (small white type), Sutter Pink (small white type), CIAT 5743 (pinto type), Zircon (small white type), Rogers Brothers D77135 (small white type), and Spinel (Great Northern type). Seeds were planted

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<sup>2</sup> Abbreviations:  $\delta$ , carbon isotope ratio;  $a$ , isotopic fractionation associated with <sup>13</sup>CO<sub>2</sub> diffusion;  $A$ , photosynthesis rate;  $b$ , carbon isotope fractionation associated with carboxylation;  $c_i$ , intercellular CO<sub>2</sub> concentration;  $c_a$ , atmospheric CO<sub>2</sub> concentration;  $E$ , transpiration rate;  $g$ , leaf conductance to water vapor;  $\Delta$ , carbon isotope discrimination.

during mid-June 1986 at the Biology Experimental Garden on the University of Utah campus. Plants (10 per cultivar) were grown at 50 cm intervals in rows 100 cm apart. All plants received adequate watering throughout the growing season (watered to field capacity at weekly intervals) and were fertilized (20:20:10, N-P-K) at sowing, early and late vegetative stages.

Leaf conductances to water vapor (upper and lower surfaces) were measured with a null balance porometer (model LI-1600, Licor Instruments). To obtain the maximum daily leaf conductances ( $g_{\max}$ ), diurnal observations were first collected. From these observations, it was clear that  $g_{\max}$  occurred between 0830 and 1100 h (MDT). Thus, in subsequent observations, different recently matured leaves (new leaves produced every 5–10 d) from each of the cultivars were continuously and sequentially sampled between these time intervals over a 3-d period to get reliable estimates of  $g_{\max}$ . Leaf conductance estimates were collected during the vegetative stage (approximately 5 weeks following planting) and again during the reproductive stage (flowering, approximately 8 weeks following planting). The Cahone cultivar was senescing before the second sampling period. None of the plants could be sampled during the late pod-filling stages because of an unexpected snow storm that curtailed the growing season in early September.

Carbon isotope ratios ( $\delta^{13}\text{C}$ ) were determined on the same sets of leaves sampled for  $g_{\max}$  estimates. Leaves were dried and ground to 40 mesh to homogenize the tissues. For whole leaf measurements, a 2 to 3 mg subsample of the tissue was combusted for 6 h at 850°C to produce  $\text{CO}_2$  in sealed, evacuated Vicor tubes containing cupric oxide and silver foil, and followed by cooling slowly to room temperature over a 12 h period. For determination of the  $\delta^{13}\text{C}$  values of cellulose, cellulose was first extracted from a subsample of the ground whole leaf material (27) and then the cellulose was combusted to produce  $\text{CO}_2$  as described earlier. The  $\delta^{13}\text{C}$  of the  $\text{CO}_2$  produced by combustion was purified cryogenically and the  $\delta^{13}\text{C}$  value of the  $\text{CO}_2$  was measured on a Finnigan MAT delta E isotope ratioing mass spectrometer. The instrument error (twice the standard deviation) associated with each observation was  $\pm 0.01\text{‰}$ , and the error between repeated analyses of the same ground tissue was less than  $\pm 0.14\text{‰}$ .

In terms of the isotopic composition (relative to the PDB standard) as typically used by geochemists,  $\delta$

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \quad (2)$$

where  $\delta_a$  is the carbon isotope ratio of atmospheric (source)  $\text{CO}_2$ . Leaf carbon isotope discrimination values ( $\Delta$ ) were calculated from  $\delta^{13}\text{C}$  values (Eq. 2), using a value for  $\delta_a$  of the  $\text{CO}_2$  of outside air of  $-8.0\text{‰}$ .

Leaf Kjeldahl nitrogen values were determined on a 10 mg subsample of the ground leaf tissues using an autoanalyser (Scientific Instruments Corp., model CFA200).

## RESULTS AND DISCUSSION

### Leaf versus Cellulose Carbon Isotope Composition

Carbon isotope discrimination values were measured on both whole leaf tissues and subsequently on the cellulose

extracted from those leaf tissues. There was a strong, significant correlation between the  $\Delta$  values measured on whole leaf tissues of different bean cultivars and those based on the extracted cellulose (Fig. 1). The slope of the relationship was not significantly different from 1 and the  $x$ -intercept has a nonzero value. The  $\Delta$  value for cellulose averaged 1.3‰ heavier than that of leaves across the entire range of leaf tissue values. Such a set of results should be expected, since there is a secondary fractionation in lipid metabolism. Lipids are much lighter in  $^{13}\text{C}$  than other leaf components (19). If other carbon-based compounds within the leaf are derived from the remaining carbon, the isotopic composition must be heavier to maintain a mass balance. Since the two different  $\Delta$  measurements were so well correlated with each other, only leaf  $\Delta$  values were measured thereafter.

The data in Figure 1 reveal substantial genetic variation in the  $\Delta$  values among highly developed dry bean lines. In the limited sampling within our experiments, bean lines differed in  $\Delta$  by approximately 2‰, which is somewhat less than the range of values reported by Ehleringer *et al.* (8). This difference in isotopic composition is equivalent to an overall  $c_i$  difference among bean lines of more than  $31 \mu\text{L L}^{-1}$ . Although extensive surveys have yet to be conducted for any single crop species, the isotopic variation in common bean is of similar magnitude to that seen in other crop species (12).

### Stability of Carbon Isotope Discrimination Measurement

There is no *a priori* basis for assuming that the differences among genetic materials are maintained through time or across developmental stages. To examine the stability of carbon isotope discrimination, we measured  $\Delta$  values on the same plants during both vegetative and reproductive developmental stages. The correlation in  $\Delta$  values across dates was highly significant ( $r = 0.89$ ,  $n = 9$ ,  $P < 0.01$ ), indicating that the rankings of genotypes measured early in the growing season were maintained through time (Fig. 2).

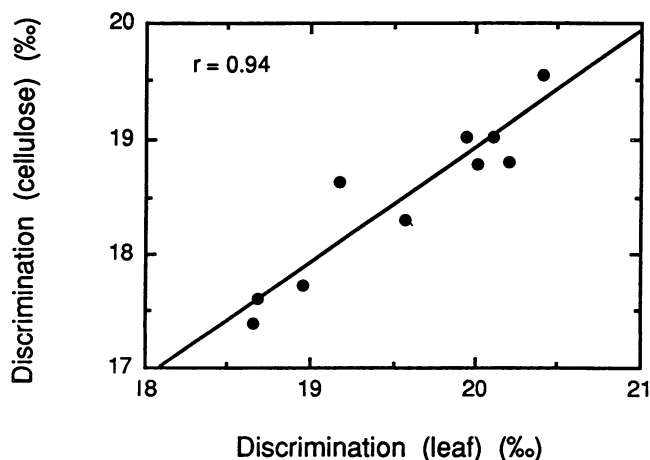
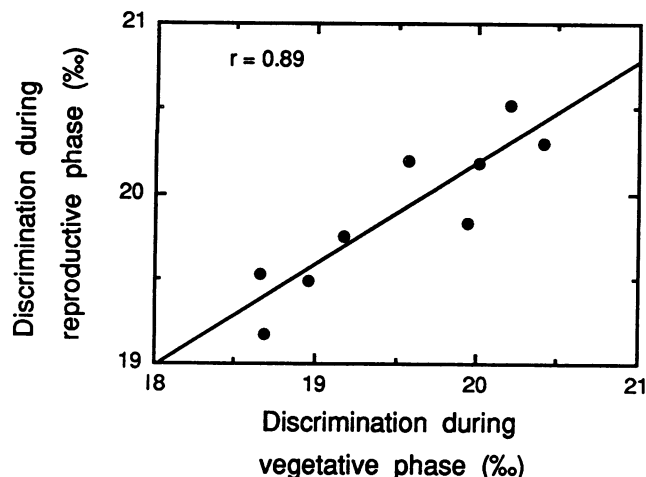


Figure 1. Correlation between carbon isotope discrimination values ( $\Delta$ ) of whole leaf material and that of the cellulose extracted from the whole leaf material for different cultivars of *P. vulgaris*.



**Figure 2.** Correlation between carbon isotope discrimination values ( $\Delta$ ) of leaf materials sampled during the vegetative phase and reproductive phases for different cultivars of *P. vulgaris*.

### Leaf Conductance and Carbon Isotope Discrimination

The main objective of our study was to examine possible relationships between leaf conductance to water vapor and  $\Delta$ . During the vegetative phase, we observed large differences in  $g_{\max}$ , despite all plants being well-watered. The  $g_{\max}$  values ranged between 459 and 630  $\text{mmol m}^{-2}\text{s}^{-1}$  during the vegetative stage. Between vegetative and reproductive growth stages, these cultivar differences in stomatal activity were maintained and  $g_{\max}$  values were significantly and positively correlated (Fig. 3).

There was a significant, positive correlation between  $g_{\max}$  and  $\Delta$  values among cultivars ( $r = 0.86$ ,  $n = 10$ ,  $P < 0.01$ ). These correlations were further examined by comparing between leaf conductance of upper and lower surfaces with  $\Delta$  (Fig. 4). In both cases, leaf conductance of adaxial and abaxial surfaces was significantly correlated with  $\Delta$  ( $P < 0.01$  for either surface).

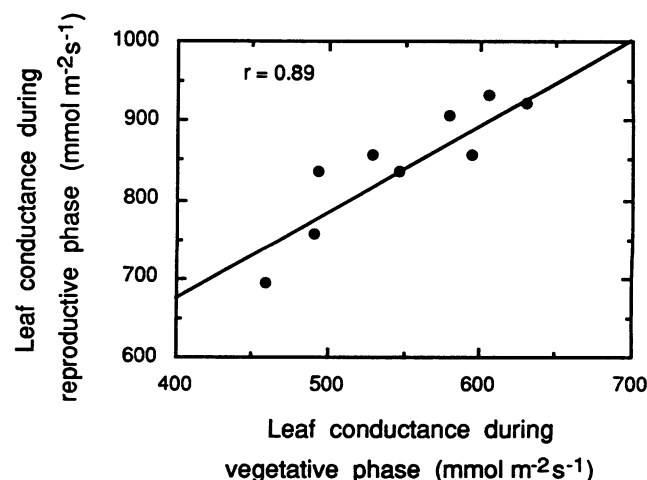
Condon *et al.* (3) and DeLucia *et al.* (4) have also reported positive correlations between leaf conductance and  $\Delta$ . However, in the wheat cultivars examined by Condon *et al.* (3), there was no significant correlation between  $g$  and  $\Delta$  for the adaxial leaf surface, which was the predominant gas exchanging surface, comprising approximately 70% of the total leaf conductance. The significant correlation between  $g_{\max}$  and  $\Delta$  was for the abaxial surface, which had a limited overall impact on leaf gas exchange rates in that species. In their study of plants in a Sierra Nevada community, DeLucia *et al.* (4) reported a positive correlation between  $g_{\max}$  and  $\Delta$  across a range of native species, but provided no information on possible intraspecific variation in this character.

Intraspecific variation in stomatal density characteristics are known for crop plants (17), and it was possible that this character may have accounted for the observed differences in  $g_{\max}$  in Figures 3 and 4. To examine this possibility, epidermal peels of upper and lower surfaces were collected on all bean cultivars. Stomatal densities among bean lines varied between 45 and 124  $\text{mm}^{-2}$  on the adaxial surfaces and between 254

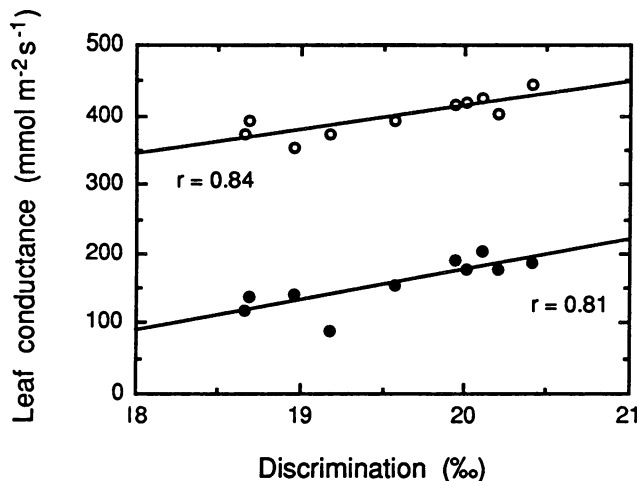
and 537  $\text{mm}^{-2}$  on the abaxial surfaces. However, there were no significant correlations between  $g_{\max}$  values and stomatal density (adaxial surface:  $r = 0.17$ ,  $n = 10$ , NS; abaxial surface:  $r = 0.09$ ,  $n = 10$ , NS; both surfaces:  $r = 0.12$ ,  $n = 10$ , NS). The basis for observed difference in  $g_{\max}$  was thus not a simple relationship with stomatal density. This result has implications for breeding programs where it is often assumed that stomatal density, an easily diagnosed character, and gas exchange rates are positively correlated (17). The lack of correlation between stomatal density and  $g_{\max}$  suggests that other factors controlling diffusion rates are more important. Most likely among these characters are intraspecific differences in the stomatal sensitivity to humidity and/or differences in the amount of a root-produced chemical messenger affecting stomatal conductance (23, 28).

Carbon isotope discrimination was not significantly correlated with leaf Kjeldahl nitrogen content at either sampling date (vegetative:  $r = -0.39$ ,  $n = 10$ , NS; reproductive:  $r = 0.44$ ,  $n = 9$ , NS). During the vegetative sample period Kjeldahl nitrogen contents averaged  $41.1 \pm 3.7 \text{ mg g}^{-1}$  ( $\bar{X} \pm \text{SD}$ ) and during the reproductive sample period  $36.7 \pm 4.7 \text{ mg g}^{-1}$ . Since both the initial slope and plateau rates of the  $A$  versus  $c_i$  response curve are positively correlated with leaf Kjeldahl nitrogen content in  $C_3$  plants (6, 9, 16, 25), a reasonable conclusion would be that the shapes of the  $A$  versus  $c_i$  response curves of the different bean lines in this study were similar.

One consequence of suggesting that the  $A$  versus  $c_i$  response curves of the different bean lines were similar is that the observed positive correlations between  $\Delta$  and  $g$  among bean lines can only be interpreted as indicating that there were differences in the extent to which stomata limit photosynthetic rate. The degree of stomatal limitation in a cultivar would be directly related to its  $\Delta$  value. Low  $\Delta$  lines would have a greater stomatal diffusion limitation than high  $\Delta$  lines. No quantitative estimates of the stomatal limitation to photosynthesis could be calculated since the photosynthetic rates at a  $c_i$  value equal to  $c_a$  were not measured in this study.



**Figure 3.** Correlation between the maximum leaf conductance to water vapor ( $g_{\max}$ ) measured during the vegetative and reproductive phenological phases for different cultivars of *P. vulgaris*.



**Figure 4.** Correlation between maximum leaf conductance to water vapor ( $g_{\max}$ ) and carbon isotope discrimination ( $\Delta$ ) of whole leaf material for different cultivars of *P. vulgaris*. Leaf conductances are plotted separately for adaxial (●) and abaxial (○) surfaces.

However, these data are suggestive that there may have been substantial genetic variation in this character.

In an earlier study, Peet *et al.* (21) described cultivar-level differences in  $g$  and  $A$  that were positively correlated with yield differences. While it should come as no surprise that photosynthetic gas exchange rates and yield are correlated, that such a clear relationship emerges without considering the role of canopy architecture in determining primary productivity rates is unusual (18). Yet leaf area indices of common beans under rainfed conditions tend to be low (LAI less than 2), and therefore single leaf-level photosynthetic rates are much more important in determining productivity rates than they would be under high LAI conditions (26). The positive correlations between  $\Delta$  and  $g$  in this study when combined with productivity and gas exchange observations of Peet *et al.* (21) and White *et al.* (26) suggest that stomatal limitations in common bean under rainfed conditions may play a significant role in limiting gas exchange rates and this is ultimately translated into a decreased growth rate.

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