

Communication

Carbon Isotope Discrimination Varies Genetically in C_4 Species

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ABSTRACT

Carbon-isotope discrimination (Δ) is used to distinguish between different photosynthetic pathways. It has also been shown that variation in Δ occurs among varieties of C_3 species, but not as yet, in C_4 species. We now report that Δ also varies among genotypes of sorghum (*Sorghum bicolor* Moench), a C_4 species. The discrimination in leaves of field-grown plants of 12 diverse genotypes of sorghum was measured and compared with their grain yields. Discrimination varied significantly among genotypes, and there was a significant negative correlation between grain yield and Δ . The variation in Δ may be caused by genetic differences in either leakiness of the bundle-sheath cells or by differences in the ratio of assimilation rate to stomatal conductance. At the leaf level, the former should be related to light-use efficiency of carbon fixation and the latter should be related to transpiration efficiency. Both could relate to the yield of the crop.

Carbon-isotope discrimination of plants with the C_3 pathway may be used to detect genetic differences in water-use efficiency of individual plants and of crops (8). Recent analysis of restriction fragment length polymorphisms from the DNA of progeny of a cross between a commercial tomato variety and a drought-resistant relative showed that as few as two genes could determine differences in water-use efficiency (18).

A number of important crops, such as corn, sorghum, and sugarcane possess the C_4 pathway of photosynthetic carbon assimilation, but no correlations between discrimination and water-use efficiency or yield have been reported for such crops. There is some evidence of interspecific variation in Δ^1 among plants possessing the C_4 pathway (20). However, within species, little variation in Δ has been observed to date. Analysis of isotope composition in 120 genotypes of *Zea mays* failed to show any significant genetic variation (21).

The mechanism of discrimination in plants possessing the C_4 pathway is such that these plants discriminate less than do those with the C_3 pathway (1, 23). Models predict that in C_4

photosynthesis isotope discrimination should depend on the ratio of mesophyll intercellular and atmospheric partial pressures of CO_2 (p_i/p_a), and leakiness (6). It may be predicted from simplified theory (6, 8) that

$$\Delta = a + (b_4 + b_3\phi - a)p_i/p_a \quad (1)$$

where Δ is defined as $R_a/R_p - 1$ and R_a and R_p are the $^{13}C/^{12}C$ molar ratios in the air and plant material, respectively (8); a is the fractionation due to diffusion in air (0.0044 [24]), b_4 is the effective fractionation by PEPCase against gaseous $^{13}CO_2$ (−0.0057 [8]), b_3 is the fractionation by Rubisco against $^{13}CO_2$ (0.030 [10, 22]) when one takes into account isotopic equilibration between gas and solution (8, 9), and ϕ is the leakiness, the proportion of CO_2 fixed by PEPCase and transported from the mesophyll to the bundle sheath which subsequently leaks back to the mesophyll.

Leakiness is also a measure of the overcycling by PEPCase compared to Rubisco, and therefore of extra light energy required for carbon fixation. This led to the suggestion that differences in Δ among C_4 species could reflect differences in leakiness and in light-use efficiency (6). At the time this was supported by data of quantum yields of carbon fixation of different C_4 types (4). Light-use efficiency can vary in sorghum (11), but no association has yet been shown with Δ .

In addition to leakiness effects on Δ , there are those generated by differences in p_i/p_a (Eq. 1). Because p_i/p_a can be affected by stomatal conductance and assimilation capacity (8), it relates to the amount of carbon fixed per unit of water used, or transpiration efficiency, and this association has been explored in C_3 species (8). In C_4 species, differences in Δ could reflect differences in light-use efficiency and/or transpiration efficiency at the leaf level.

Since growth in closed crop canopies can be limited by light absorption or water availability, small increases in light-use efficiency or transpiration efficiency at the leaf level could translate into increases in dry matter production and affect grain yield. We report that there is genetic variation in Δ in sorghum, a C_4 species, and that there is also correlation between grain yield and Δ for genotypes of this C_4 species when grown in a field experiment.

¹ Abbreviations: Δ , carbon-isotope discrimination; PEPCase, phosphoenolpyruvate carboxylase; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase (E.C. 4.1.1.39); FL, flag leaf; T, metric ton.

MATERIALS AND METHODS

Twelve genotypes of sorghum (*Sorghum bicolor* Moench, Table I) were grown in two field experiments conducted at Emerald Research Station in central Queensland (lat. 23° S., long. 148° 05' E). Both experiments were planted February 3, 1988, on an 80 cm deep, uniform, cracking clay soil using randomized block designs with three replicates. One experiment included the four genotypes Texas 610 SR, Goldrush II, DK 55+, and Guardian MR, planted at a density of 70,000 plants ha⁻¹. The other experiment, which also included the remaining eight genotypes (Table I), was planted at a density of 90,000 ha⁻¹. Both experiments were planted in 80 cm wide rows, and experimental units four rows wide by 12 m long were established. Time of flowering was recorded as the day that at least half of the plants in a plot had exerted anthers midway down the panicle of the main culm. Pests and diseases were controlled as required. To determine grain yield, panicles of plants in the central 9.9 m of the middle two rows in each plot were hand harvested on June 27, 1988, and the grain separated from the panicles.

Samples of the FL and the leaf third from the FL (FL-3) of 10 plants in each of the three replicate plots per genotype were collected shortly before harvest. The dried material of the ten leaves of each type from each plot was combined and finely ground to a mean particle size of 100 μm. A subsample of 5 to 10 mg from the ground material was analyzed for carbon isotope composition with a modified model 1106 Carlo Erba elemental analyzer interfaced to a VG Isogas SIRA 24 ratio mass spectrometer. The isotope ratio of each sample was determined by comparison with a working standard of CO₂ gas which, in turn, was calibrated against CO₂ generated from international limestone standards and had an isotope ratio -35.08‰ relative to PeeDee Belemnite (PDB). The performance of the mass spectrometer was monitored by replicated analyses of a pure sample of ANU sucrose with isotope composition -10.50‰ relative to PDB. A run of plant samples interspersed with 10 sucrose samples resulted in a range of 0.1‰ in the sucrose isotope compositions and a standard deviation of 0.03‰, indicating good reproducibility of the combustion system/mass spectrometer. For calculation of discrimination, the isotope composition of CO₂ in the air was assumed to be -7.60‰ relative to PDB (16). Discrimination was calculated (14) using

$$\Delta = \frac{-7.60 \times 10^{-3} - \delta_p}{1 + \delta_p}$$

Data were analyzed using a two-way analysis of variance and correlation with grain yield was calculated.

RESULTS AND DISCUSSION

The genetic variation in Δ was small, with a range of 0.6×10^{-3} in genotype means, but the variation was significant (Table I). The sampling strategy, in which a plot of plants of one genotype was considered an experimental unit, minimized errors due to small plant-to-plant variations in isotope compositions. The strategy was to combine 10 leaves, each from a different plant in a plot, for grinding and isotope analysis. Thus plant-to-plant variation in isotope discrimina-

Table I. Carbon-Isotope Discrimination (Δ) in Dry Matter of the Flag Leaves (FL), the Third Leaf from the Top of the Plant (FL-3), and the Mean Discrimination for the Leaves, Grain Yield, Harvest Index, and Time-to-Flowering of 12 Sorghum Genotypes Grown in the Field

Least significant difference for comparisons of Δ among cultivar means = 0.013 ($P = 0.05$) and 0.018 for comparisons among means for leaves and genotypes.

Cultivar	Discrimination $10^3 \times \Delta$			Yield T/ha	Time to flowering d
	mean	leaf FL-3	FL		
Goldrush II	4.84	4.80	4.87	1.94	58
Texas 610SR	4.83	4.83	4.83	2.40	63
Nugget II	4.72	4.78	4.65	2.26	57
White Charger	4.65	4.66	4.64	2.93	67
Guardian MR	4.62	4.52	4.72	3.40	71
DK 55+	4.57	4.53	4.60	2.99	68
E57+	4.56	4.51	4.61	3.23	68
Hylan 4 × 8	4.50	4.47	4.53	3.47	63
Goldmine	4.47	4.36	4.59	3.52	62
Pride	4.46	4.35	4.58	3.17	62
DK 470	4.38	4.27	4.48	2.80	76
6990/16-6	4.24	4.05	4.42	2.86	74

tion was averaged over a single plot, and the means in Table I are from three samples per genotype. The coefficient of variation of this data set indicated that the variation in Δ around the mean was 0.1×10^{-3} . Similar sampling strategies have indicated small, but significant, genetic variation in Δ in C₃ species (3, 7, 13–15, 19, 26). For example, the range in mean genotype Δ was just 0.9×10^{-3} in leaves of a group of 16 peanut genotypes but Δ was significantly different among genotypes (15).

There were significant differences in Δ among the plant parts with FL and FL-3 leaves having differing values. The ranking of Δ in the different-aged leaves varied significantly so that the ranking depended on the leaf chosen. However, this change in ranking depended mostly on the variation in Δ of the leaves in just three genotypes (Pride, Goldmine, and Guardian MR). Regardless of the leaf chosen for measurement of Δ , grain yield and Δ were negatively correlated.

Figure 1 shows grain yield plotted against the average discrimination in the two leaves. A quadratic regression of grain yield on Δ explained more of the variation ($r^2 = 0.67$) than did a linear regression ($r^2 = 0.29$). The improvement of fit occurred because the values of grain yield of two of the genotypes, 6990/16-6 and DK 470, were lower than expected, given their small values of Δ (Fig. 1). These two genotypes flowered late (d 76 and 74), and when excluded from the analysis, a strong linear relationship between grain yield and Δ ($r^2 = 0.79$) was obtained. Departure from linearity for these two late-maturing genotypes was associated with increasing moisture stress between effective falls of rain on d 60 and 88. The increasing stress adversely affected the grain number and grain yield of these late genotypes.

The range in Δ was similar to the 0.8‰ difference found between two genotypes of *Panicum coloratum* (20). Apart from that report, this is the first, of which we are aware, to show significant genetic variation in discrimination among genotypes in one C₄ species and certainly the first for a range

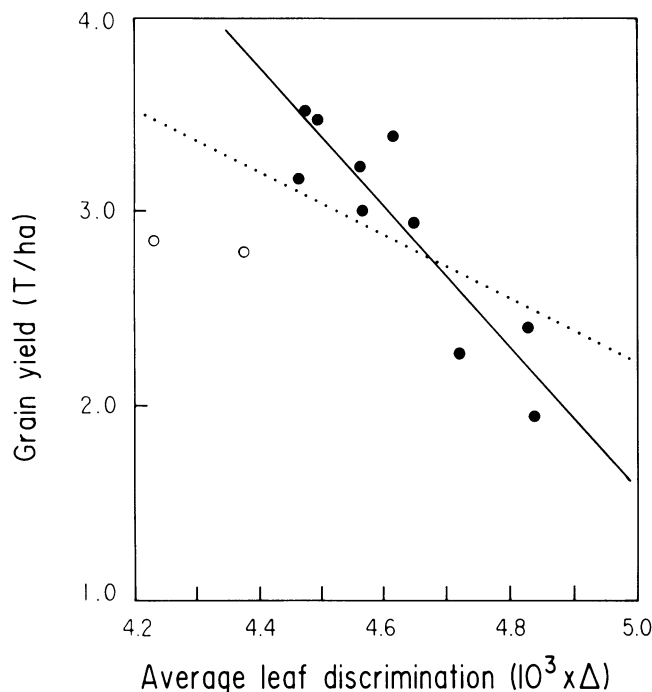


Figure 1. Relationship between grain yield of 12 diverse genotypes of sorghum and the mean isotope discrimination measured in the leaves of the genotypes. Solid symbols are for the genotypes which flowered in 71 d or less. Open symbols are for the genotypes which flowered in 74 and 76 d. Regression equation for yield on mean discrimination of 10 early-flowering genotypes: $y = 19.7 - 3.6x$, ($r^2 = 0.79$), represented by the solid line. Regression equation for yield on mean discrimination of all 12 genotypes; $y = 10.3 - 1.6x$, ($r^2 = 0.29$), represented by dotted line.

of genotypes. In contrast, O'Leary (21) reported no significant differences in discrimination among 120 lines of maize. We chose diverse sorghum genotypes, and this may explain the significant variation in Δ found here.

From Equation 1, it appears that there are two main ways by which Δ could have changed, *i.e.* changes in p_i/p_a or in ϕ . In the former case, we would expect changes in transpiration efficiency to be associated with those in Δ , at least at the level of individual plants. In C_3 species, this correlation would be a negative one, but for C_4 species, the correlation could be either negative or positive, depending on whether ϕ is greater or less than $(a-b_4)/b_3$, which is approximately 0.34. The relationship between Δ and p_i/p_a in C_4 species, as determined from simultaneous measurements of discrimination and gas exchange, has normally been one of independence or a negative dependence of Δ on p_i/p_a (2, 5, 9, 25) except when plants were water- or salt-stressed (2). A value of ϕ less than 0.34 is inferred from the negative dependence.

If the difference in Δ resulted from variation only in p_i/p_a , a change of 0.6×10^{-3} would correspond to reasonably large changes in stomatal conductance or in photosynthetic capacity. Such differences should be sufficiently large to be measured by conventional gas-exchange techniques. Variation has been observed in stomatal conductance (12), assimilation rate, and p_i/p_a (17) among genotypes of sorghum. If we assume that ϕ was less than 0.34 and that most of the carbon measured

in the present experiments was accumulated when the plants were not stressed, then smaller values of Δ associated here with higher yield would be associated with larger p_i/p_a (caused either by greater conductance, or smaller photosynthetic capacity) as has been reported for wheat (3). Greater conductance might be linked with greater yield because assimilation would increase as greater conductance allowed more CO_2 to diffuse into the leaf and saturate the biochemical reactions of photosynthesis. However, smaller photosynthetic capacity (for a constant leaf conductance) could also be linked to yield, if it were caused by an increase in the rate of leaf expansion. A high rate of leaf expansion could result in an increased value of p_i/p_a if the photosynthetic capacity were diluted by growth. If, on the other hand, the leakiness (ϕ in Eq. 1) was the source of variation observed here in Δ , we would expect a negative relationship between the quantum yield of photosynthetic carbon assimilation and Δ . The fact that yield and Δ were negatively correlated is consistent with this and could reflect variation in leakiness and light-use efficiency. The small range in Δ that we observed would correspond to a small variation in ϕ (in fact 0.05) if that were the source.

Irrespective of the source of variation in gas-exchange processes that gave rise to the variation in Δ , the correlation between yield and Δ should be pursued for this and other C_4 species. The relationship may be complex, but there are grounds for optimism that the molecular basis for the genetic variation in Δ may be determined using the techniques of restriction fragment length polymorphism analysis as is successfully being done with C_3 species (18).

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