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Photosynthetic Characteristics of Segregates from Hybrids between *Flaveria brownii* (C_4 Like) and *Flaveria linearis* (C_3-C_4)[1]

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Characteristics related to C_4 photosynthesis were studied in reciprocal F_1 hybrids and F_2 plants from *Flaveria brownii* (C_4 like) and *Flaveria linearis* (C_3-C_4). The reciprocal F_1 plants differed in $^{13}C/^{12}C$ ratios of leaves and the percentage of ^{14}C initially incorporated into C_4 acids, being more like the pollen parents in these traits. They did not differ in apparent photosynthesis or in O_2 inhibition of apparent photosynthesis and differed only slightly in CO_2 compensation concentration at 175 μmol quanta m^{-2} s^{-1} and 400 mL L^{-1} O_2. The $^{13}C/^{12}C$ ratios of 78 F_2 progeny from the two F_1 plants exhibited a normal distribution centered between those of the parents, with a few values slightly higher and lower than the parents. Apparent photosynthesis at 130 μL L^{-1} CO_2 and inhibition of photosynthesis by O_2 was nearly normally distributed in the F_2 population, but no values for F_2 plants approached those for *F. brownii* (15.4 μmol m^{-2} s^{-1} and 7.8%, respectively). Distribution of the CO_2 compensation concentration measured at 1000 μmol quanta m^{-2} s^{-1} and 400 mL L^{-1} of O_2 in the F_2 population was skewed toward *F. brownii* with 72% of the progeny having values <9 μL of CO_2 L^{-1} compared to 1.5 and 27.2 μL L^{-1} for *F. brownii* and *F. linearis*, respectively. Correlations among traits of F_2 plants were low (coefficients of 0.30 to −0.49), indicating that the C_4-related traits are not closely linked in segregating populations. Plants in the F_2 population selected for high or low apparent photosynthesis at 130 μL of CO_2 L^{-1} (six each) did not rank consistently high or low for $^{13}C/^{12}C$ ratios, O_2 inhibition of apparent photosynthesis, CO_2 compensation concentration, or activities of phospho*enol*pyruvate carboxylase or NADP-malic enzyme. This study confirms results of earlier work that indicates independent segregation of C_4 traits and also shows that the C_4-like parental type can be recovered, at least for some characteristics ($^{13}C/^{12}C$ ratio), in segregating populations. Recovery of fully functional C_4 plants awaits further experimentation with $C_4 \times C_3$ or $C_4 \times C_3$-C_4 hybrid plants that produce fertile progeny.

Because C_4 photosynthesis confers on plants adaptive advantages in certain habitats, it is important to study inheritance of traits associated with this pathway. There are a very limited number of taxa known that afford the opportunity for genetic studies of C_4 traits. Hybrids between species differing in the degree of C_4 photosynthesis have been made in only two genera, *Atriplex* and *Flaveria*. Hybrids between C_3 and C_4 species in *Atriplex* resulted in F_1, F_2, and F_3 progeny more like the C_3 than the C_4 parent (Björkman et al., 1971; Hinata et al., 1984). Segregates in F_2 and F_3 populations differed in some C_4-related traits, but none of the segregates approached the C_4 parent in functionally coordinated C_4 photosynthesis (Björkman et al., 1971). O_2 inhibition of *AP* and Γ values of F_2 and F_3 progeny indicated mainly C_3 photosynthesis in the hybrids. Abnormal chromosome behavior in the hybrid progeny probably affected inheritance of traits.

Several interspecific hybrids have been made in *Flaveria*. Performance of F_1 plants has been variable; in some cases traits have been similar to means halfway between those of the parents, whereas in others they have been similar to the more C_3-like parent (Holaday et al., 1985; Brown et al., 1986; Holaday et al., 1988; Apel et al., 1988, 1989). It appears that in some hybrids of *Flaveria*, as opposed to *Atriplex*, the photosynthetic carbon assimilation (mesophyll) and carbon reduction (bundle sheath) portions of the C_4 cycle are coordinated well enough to reduce photorespiration and allow a C_4 cycle to assimilate a portion of the CO_2 (Byrd et al., 1992).

Investigations of inheritance of C_4 traits in *Flaveria* beyond the F_1 generation have been very limited. In a small F_2 population (31 plants) from *Flaveria brownii* (C_4 like) × *Flaveria linearis* (C_3-C_4), $\delta^{13}C$ values were between those of the parents (−27.3 to −20.5‰) and averaged −24.6‰ compared with −24.7‰ for the F_1 plant (Huber et al., 1989). On the other hand, Γ measured at low irradiance for the F_1 (6.5 μL L^{-1}) was nearer the value for *F. brownii* (4.0 μL L^{-1}) than for *F. linearis* (34.0 μL L^{-1}), whereas the F_2 plants ranged from 3.7 to 11.7 μL L^{-1}. Keefe (1987) produced several reciprocal F_1 hybrids from *F. brownii* and *Flaveria floridana* (C_3-C_4) and made reciprocal backcrosses with one of the F_1 plants and *F. brownii*. Values of $\delta^{13}C$ of the F_1 and backcross plants were between those of the parents, as were several anatomical features. It was concluded that no maternal effects were present for any of the traits and that additive, codominant genetic loci were involved with at least three or four independently segregating factors influencing $\delta^{13}C$ and anatomical characters. When *Flaveria cronquistii* (C_3) was hybridized

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Abbreviations: *AP*, apparent photosynthesis; AP_{130}, apparent photosynthesis measured at 130 μL L^{-1} of CO_2; BSC, bundle sheath cells; *Ci* (*Ca*), intercellular (ambient) CO_2 concentrations; Γ, CO_2 compensation concentration; $\delta^{13}C$, $^{13}C/^{12}C$ ratio of leaves relative to ratio of a standard; NADP-ME, NADP-dependent malic enzyme; PEPcase, phospho*enol*pyruvate carboxylase.

with *Flaveria palmeri* (C_4 like), the resulting $\delta^{13}C$ value was −25.3‰, very similar to that of *F. cronquistii* (−25.6‰) (Apel et al., 1988). Backcrossing of the F_1 hybrid onto *F. palmeri* produced a plant with a similar $\delta^{13}C$ value (−25.9‰), but a further backcross of this plant with *F. palmeri* produced a plant with a C_4-like $\delta^{13}C$ value (−16.0‰). Likewise, hybridization of the F_1 plant *F. brownii* × *F. linearis* with *Flaveria trinervia* (C_4) gave more positive $\delta^{13}C$ values (−20.5 and −23.2‰ in two hybrids) compared to −27.3‰ for *F. brownii* × *F. linearis* (Byrd et al., 1992). These results indicate that in *Flaveria* more C_4-like plants can be produced by backcrossing and by selection in segregating populations.

Characterization of large segregating populations for traits related to C_4 photosynthesis has not been reported, although such populations are likely to be useful in inheritance studies. In this paper we report photosynthetic characteristics of F_1 and F_2 plants from reciprocal hybrids between *F. brownii* and *F. linearis*.

MATERIALS AND METHODS

Plant Culture and Hybridization

Flaveria brownii AM Powell (85–212) and *Flaveria linearis* Lag. (85–26) were crossed in a reciprocal way as described by Huber et al. (1989). The F_1 plants from one reciprocal set of crosses described in that paper were selfed to provide F_2 progeny for evaluation of segregation of traits. Florets were self-pollinated by rubbing heads between the thumb and forefinger, and crossing was prevented by covering individual inflorescences with paper bags.

The F_2 plants, along with F_1 and parent plants, were grown in an unshaded greenhouse with maximum temperatures of about 30 to 35°C at midday and minimum temperatures of 20 to 22°C at night. Plants were potted in 3-L pots in a 3:2:1 (v/v) mixture of peat, soil, and sand. Supplemental lighting was provided for 12 h during the winter and during cloudy periods to ensure an irradiance of at least 1000 μmol quanta m^{-2} s^{-1} (PPFD) during midday. Plants were vegetatively propagated and repotted when necessary to ensure vigorous vegetative growth during periods of measurement. Plants of the F_1 and F_2 generations were vigorous and normal in appearance.

CO_2 Exchange

The F_2 populations were screened by measuring CO_2 uptake on one of the youngest fully expanded leaves on the main stem or a prominent branch. To increase differentiation among plants differing in the degree of C_4 photosynthesis, CO_2 uptake was measured at low [CO_2] (130 μL L^{-1}) and high [O_2] (400 mL L^{-1}). Leaves were enclosed in small acrylic plastic chambers, and CO_2 exchange was measured as described earlier (Brown et al., 1986). Irradiance was maintained at 2 mmol quanta m^{-2} s^{-1} (PPFD) and leaf temperature at 30 ± 1°C. Water vapor pressure deficit of leaf-chamber air ranged from 16 to 23 mbar. Photosynthesis at 130 μL L^{-1} of CO_2 and 400 mL L^{-1} of O_2 was measured on 58 F_2 plants from the *F. brownii* × *F. linearis* hybrid and on 20 F_2 plants of the reciprocal hybrid. Transpiration was also measured, and leaf conductance to CO_2 (stomatal plus boundary layer) was calculated. Estimates of *Ci* were made as described earlier (Brown et al., 1986). Measurements were made at least twice on each F_2 plant and in triplicate on *F. brownii*, *F. linearis*, and the F_1 plants.

Single measurements of Γ and O_2 inhibition of *AP* were made on 54 plants from the same F_2 populations (40 from *F. brownii* × *F. linearis* and 14 from the reciprocal F_1). Inhibition of *AP* by O_2 was measured in the same way and under the same conditions as AP_{130} except that [CO_2] in the leaf chamber was 340 μL L^{-1}, and measurements were made first at 210 and then at 20 mL L^{-1} of O_2. Measurements of Γ were made with the syringe technique described earlier (Brown et al., 1985). Detached leaves were placed in 30-mL syringes, and the syringes were flushed with gas containing 100 μL L^{-1} of CO_2 and 400 mL L^{-1} of O_2 and incubated at 30°C and 1000 μmol quanta m^{-2} s^{-1} (PPFD) for 30 min. The residual CO_2 in the syringe was analyzed by injecting 20 mL of air into a N_2 stream passing through an IRGA.

^{13}C Analysis

Leaves on which AP_{130} was measured were dried at 80°C overnight. The leaves were ground to a fine powder and analyzed for $^{13}C/^{12}C$ ratio by MS in the laboratory of Dr. Barry Osmond, Duke University, Durham, NC. The $^{13}C/^{12}C$ ratios are expressed as $\delta^{13}C$ (‰). $\delta^{13}C = [(R \text{ sample}/R \text{ standard}) - 1] \times 1000$, R being the $^{13}C/^{12}C$ ratio. The standard ratio is that of Pee Dee belemnite.

Characterization of Selected F_2 Plants

Several plants were selected for further study from among the highest and lowest of the F_2 population based on AP_{130} and $\delta^{13}C$. Measurements of AP_{130} and O_2 inhibition of *AP* were repeated as described above. Measurements of Γ were repeated as described, except that a low PPFD (175 μmol quanta m^{-2} s^{-1}) was used in an attempt to increase differences among the F_2 plants. This level of irradiance has been shown to increase Γ in C_3-C_4 species somewhat but to have little effect on Γ of C_3 and C_4 species (Brown and Morgan, 1980; Holaday et al., 1982). Response of *AP* to *Ci* was determined on six F_2 plants with high and low AP_{130}, as well as *F. brownii*, *F. linearis*, and one of the F_1 plants. Measurements were made at a *Ca* of 55, 130, 240, and 350 μL L^{-1}, 400 mL L^{-1} of O_2, and 30°C leaf temperature. A high [O_2] of 400 mL L^{-1} was used to increase differences among segregates.

Leaves were pulse labeled with $^{14}CO_2$ to investigate the initial incorporation of ^{14}C in C_4 acids. Six F_2 plants were used, two each with high (−21.9 and −25.5‰), medium (−27.8 and −28.4‰), and low (−32.4 and −34.2‰) $\delta^{13}C$ values. In addition, *F. brownii*, *F. linearis*, and the two F_1 plants were labeled. Leaves were exposed to $^{14}CO_2$ for 10 s and quickly frozen in liquid N_2. Labeling and processing of samples were as described by Byrd et al. (1992).

Activities of PEPcase and NADP-ME were assayed on young, fully expanded leaves by procedures described by Cameron et al. (1989). Leaves of the parents (*F. brownii* and *F. linearis*), the reciprocal F_1 plants, and selected F_2 plants were assayed. Chl was extracted as described by Moran and Porath (1980) and assayed according to the method of Ins-

keep and Bloom (1985). Enzyme activities and Chl were normalized to a fresh weight basis so that activities could be expressed per mg of Chl. *Flaveria trinervia* (accession 84–10) was included in the measurements of enzyme activity as a C_4 control.

RESULTS

F_1 Plants

For some characteristics, the reciprocal F_1 hybrids were similar to each other (Table I). *AP* at both 130 and 350 μL L^{-1} of CO_2 was identical for the two hybrids, as was O_2 inhibition of *AP*. Activity of PEPcase was also similar in the two F_1 plants, whereas NADP-ME activity was somewhat higher for *F. brownii* × *F. linearis* (Fig. 2). On the other hand, $\delta^{13}C$ was very different, with values being similar to the respective pollen parents. The percentage of ^{14}C in C_4 acids after 10-s labeling with $^{14}CO_2$ differed between the F_1 plants, with the higher percentage in the F_1 exhibiting the higher $\delta^{13}C$ value. Values of Γ measured at 1000 μmol quanta m^{-2} s^{-1} and 400 mL L^{-1} of O_2 were similar in the two F_1 hybrids (Fig. 1C), but at low irradiance and 400 mL L^{-1} of O_2, Γ was slightly higher for the *F. brownii* × *F. linearis* hybrid (Table I). Photosynthesis at low CO_2 and Γ for the F_1 plants were closer to *F. brownii* than *F. linearis*; however, O_2 inhibition of *AP* (Table I) and activities of PEPcase and NADP-ME (Fig. 2) were more like that of *F. linearis*.

Activities of PEPcase and NADP-ME in *F. brownii* and *F. linearis* were lower than reported earlier (Ku et al., 1983; Cheng et al., 1989). These enzymes also had lower activities in *F. trinervia* (Fig. 2) than reported for most experiments (Ku et al., 1983) but were similar to values reported by Bauwe (1984) and Moore et al. (1989). Although we do not know the reason for the lower activities in our experiments, we consider it unlikely that the ranking among parents and progeny is different than would occur at higher activities.

Segregation of Traits in F_2 Plants

The frequency distribution of $\delta^{13}C$, AP_{130}, and O_2 inhibition of *AP* followed near-normal curves (Fig. 1). The greatest frequency of plants for $\delta^{13}C$ and AP_{130} was near the mean of the parental species, whereas for O_2 inhibition of *AP* the greater frequencies were nearer to *F. linearis*. In contrast, Γ for the F_2 population was close to *F. brownii* with 72% of the plants having values <9 μL L^{-1}.

For both δ ^{13}C and Γ, F_2 plants covered the range of the parents, and for $\delta^{13}C$, three plants were more negative than *F. linearis* (−30.9‰) and three were more positive than *F. brownii* (−23.5‰). However, no values of AP_{130} were as high in the F_2 population (maximum was 13.4 μmol of CO_2 m^{-2} s^{-1}) as for *F. brownii* (15.4 μmol m^{-2} s^{-1}), and no values of O_2 inhibition of *AP* were as low (minimum was 11.2%) as for *F. brownii* (7.8%).

Correlations among traits for the F_2 plants were not high (Table II). The highest correlation was between *Ci/Ca* calculated from AP_{130} measurements and $\delta^{13}C$ ($r = -0.49$). No other correlation coefficient was greater than 0.33 (for *Ci/Ca* versus AP_{130}), although four were significant ($P < 0.05$), ranging from 0.27 to 0.30 (ignoring signs).

Characteristics of Selected F_2 Plants

There was variation in the responses of *AP* by F_2 plants to increasing *Ci* as shown in Figure 3 for two of the six plants tested. Progeny 87–567 had an initial slope similar to that of *F. brownii* and the F_1 (*F. brownii* × *F. linearis*), and *AP* at all *Ci* was higher than for 87–590 and *F. linearis*. Although *AP* at a *Ci* of about 250 μL L^{-1} was similar for 87–590 and *F. linearis*, the initial slope of the *AP* versus *Ci* curve was greater and Γ was lower for 87–590 than *F. linearis*. The average AP_{130} values for the six plants selected from the two extremes of the F_2 population were 11.1 ± 1.9 and 7.7 ± 1.2 μmol m^{-2} s^{-1}, but plants from the extremes did not differ consistently for the other traits examined (Table III). Although mean values for Γ and O_2 inhibition of *AP* (data not shown) were lower and $\delta^{13}C$ was higher for the high-AP_{130} group, there were overlapping data for the two groups for every trait. One F_2 plant (87–608) had an unusually high Γ value of 61 ± 4 μL L^{-1}, but other measured traits were not unusual. This plant died, so no further characterization was possible. Activities of PEPcase and NADP-ME were not correlated among the selected F_2 plants (Fig. 2). Activities of PEPcase (average of 17 progeny = 49 ± 18 μmol mg^{-1} of Chl h^{-1}) tended to be nearer to *F. linearis* (42 μmol mg^{-1} of Chl h^{-1}), and none

Table I. *Photosynthetic traits of reciprocal F_1 hybrids between F. brownii and F. linearis*
Means (±SD) are given, except for $\delta^{13}C$, which represents only one measurement per genotype.

Trait	*F. brownii*	*F. linearis*	*F. brownii* × *F. linearis*	*F. linearis* × *F. brownii*
AP (μmol m^{-2} s^{-1})[a]	35 ± 3	31 ± 2	30 ± 4	29 ± 2
AP_{130} (μmol m^{-2} s^{-1})[b]	15.4 ± 0.4	5.8 ± 1.2	12.5 ± 1.2	12.5 ± 2.8
Γ (μL L^{-1})[c]	14.7 ± 1.2	53.0 ± 6.1	22.0 ± 4.6	16.0 ± 3.5
O_2 inhibition of AP (%)[d]	7.8 ± 1.2	25.4 ± 1.8	20.1 ± 1.5	20.8 ± 2.8
δ ^{13}C (‰)	−23.5	−30.9	−30.9	−24.9
^{14}C in C_4 acids (%)[e]	92 ± 7	23 ± 1	58 ± 3	76 ± 1

[a] Measured at 30°C, 2 mmol quanta m^{-2} s^{-1} PPFD, 350 μL L^{-1} of CO_2 and 210 mL L^{-1} of O_2. [b]Measured at 30°C, 2 mmol quanta m^{-2} s^{-1} PPFD, 130 μL L^{-1} of CO_2 and 400 mL L^{-1} O_2. [c]Measured at 30°C, 175 μmol quanta m^{-2} s^{-1} PPFD, and 400 mL L^{-1} O_2. [d]Inhibition of AP by 210 compared with 20 mL L^{-1} of O_2 under conditions specified in footnote a. [e]Percentage of ^{14}C in C_4 acids after a 10-s $^{14}CO_2$ exposure.

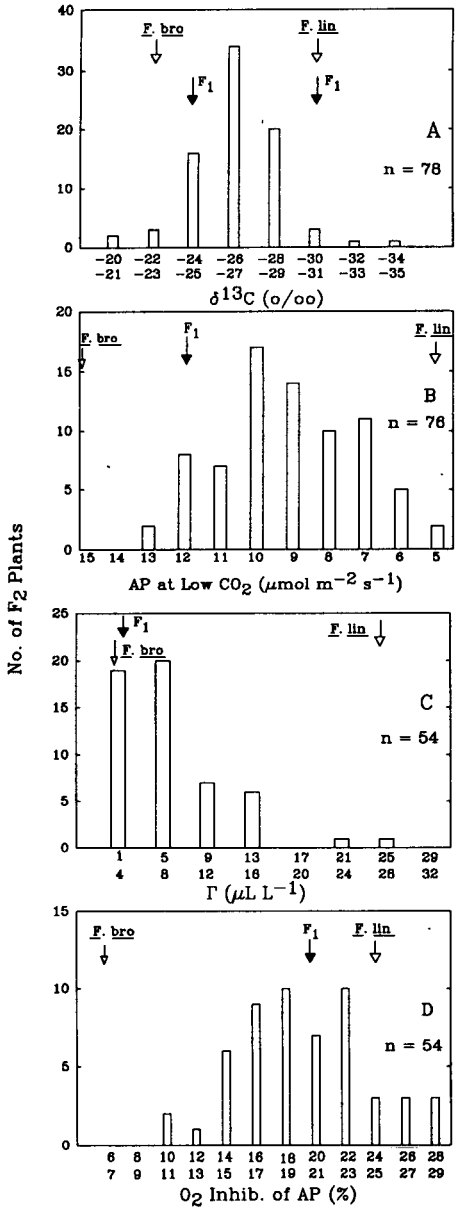

Figure 1. Frequency distribution of $\delta^{13}C$ (A), AP_{130} (B), Γ (C), and O_2 inhibition of *AP* (D) for combined F_2 progeny from reciprocal F_1 hybrids between *F. brownii* and *F. linearis*. AP_{130} and Γ were determined at 30°C, 1 mmol quanta m^{-2} s^{-1} PPFD, and 400 mL L^{-1} of O_2. Inhibition of *AP* by 210 mL L^{-1} of O_2 relative to *AP* at 20 mL L^{-1} was determined at the same temperature and irradiance and at 340 μL L^{-1} of CO_2. Open and closed arrows indicate values for the parents and F_1 hybrids, respectively.

approached *F. brownii* (173 μmol mg^{-1} of Chl h^{-1}). Two F_2 progeny whose NADP-ME activity approximated that of *F. brownii* (170 μmol mg^{-1} of Chl h^{-1}) exhibited the lowest and highest values of AP_{130}. Correlations among traits for the 12 F_2 plants (Table III) were not significant ($P < 0.05$). If 87–608 was excluded, the coefficient for the correlation between Γ and $\delta^{13}C$ for the remaining 11 plants was −0.79 and significant ($P < 0.05$). Thus, correlation among traits was low both

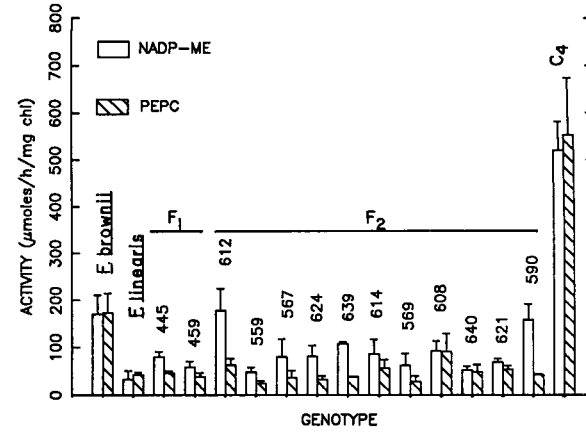

Figure 2. Segregation of NADP-ME and PEPcase (PEPC) activities among F_2 plants. *F. brownii*, *F. linearis*, and the reciprocal F_1 hybrids *F. brownii* × *F. linearis* (445) and *F. linearis* × *F. brownii* (459) are included. *F. trinervia* is also included as a C_4 control. The F_2 plants indicated for each pair of bars are those listed in Table III except 87-570, for which enzymes were not assayed. One SD is indicated above each bar.

in the overall F_2 population (Table II) and in the selected population.

Six F_2 plants selected for high, low, and medium $\delta^{13}C$ were labeled with $^{14}CO_2$ for 10 s. The two plants with high $\delta^{13}C$ values (87–612 and 87–559) also had high AP_{130} and initially incorporated 76 and 83% of ^{14}C into C_4 acids. The other four plants ranged in $\delta^{13}C$ from −27.8 to −34.2‰ and in AP_{130} from 5.7 to 9.1 μmol m^{-2} s^{-1}, but the initial incorporation of ^{14}C in C_4 acids differed little (48–57%). None of the six selected F_2 plants approached the low percentage of ^{14}C in C_4 acids exhibited by *F. linearis* (23%).

DISCUSSION

The difference between the reciprocal F_1 hybrids in $\delta^{13}C$ and to a lesser extent for other traits (Table I) does not indicate maternal inheritance; the F_1 plants were more like the respective male parents. Keefe (1987) observed considerable variability in $\delta^{13}C$ among reciprocal F_1 hybrids (10 of each) between *F. brownii* and *F. floridana* and concluded that there

Table II. *Correlations among traits of F_2 plants*

Traits	*r*	*n*
AP_{130} vs $\delta^{13}C$	0.30**[a]	78
O_2 inhibition of *AP* vs $\delta^{13}C$	−0.28*	54
Γ vs $\delta^{13}C$	−0.07	54
AP_{130} vs O_2 inhibition of *AP*	−0.16	54
Ci/*Ca* vs $\delta^{13}C$	−0.49**	70
AP_{130} vs Γ	−0.27*	54
O_2 inhibition of *AP* vs Γ	−0.29*	54
AP_{130} vs *AP* (350 μL L^{-1} of CO_2)	0.20	54
Ci/*Ca* vs AP_{130}	−0.33**	70

[a] Single or double asterisks (*, **) indicate significance at the 0.05 and 0.01 levels of probability, respectively.

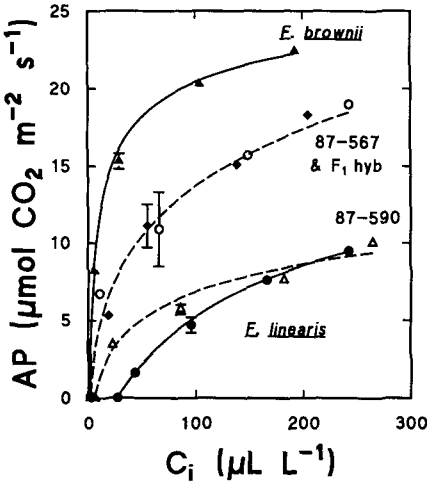

Figure 3. Response of *AP* to *Ci* for *F. brownii*, *F. linearis*, their F_1 hybrid, and two extreme F_2 plants. Data are averages of two measurements made on separate plants at 30°C, 400 mL L^{-1} of O_2, and a PPFD of 1 mmol quanta m^{-2} s^{-1}. ▲, *F. brownii*; ●, *F. linearis*; ◆, F_1 hybrid; O, F_2 plant 87-567; △, F_2 plant 87-590. Points at Γ (zero *AP*) were determined by the syringe technique described in the text. Vertical bars representing ±SD are given for *AP* at *Ci* values corresponding to a *Ca* of 130 μL L^{-1}.

was no cytoplasmic effects on $\delta^{13}C$. The reciprocal effect observed in our study is believed to be due to differing nuclear gene combinations resulting from crossing of heterozygous parents. These species are self-incompatible (Powell, 1978) and, therefore, are likely to be highly heterozygous. This heterozygosity is likely the reason for the variability of traits of *Flaveria* F_1 hybrids produced by several species combinations (Smith and Powell, 1984; Brown et al., 1986; Cameron et al., 1989; Huber et al., 1989), although there appears to be some dominance of the C_3 genome in some crosses in which hybrids have been C_3-like (Holaday et al., 1985; Apel et al., 1988; Holaday et al., 1988; Apel et al., 1989). Even in those C_3-like hybrids, however, some traits were more C_4 like. For example, Holaday et al. (1988) and Apel et al (1989) found that NADP-malate dehydrogenase activity in reciprocal *F. brownii* × C_3 hybrids was similar to that in *F. brownii*. Alanine aminotransferase activity was 120% of the parental mean in a hybrid between *F. palmeri* (C_4 like) and *F. cronquistii* (C_3) (Apel et al., 1988).

The several traits measured in this work represent partial estimates of the degree of C_4 photosynthesis. The $\delta^{13}C$ values are probably most indicative of the functional completeness of the C_4 cycle, i.e. the degree to which the C_4 cycle accounts for the CO_2 assimilated. It is, therefore, instructive about genetics of the C_4 cycle that $\delta^{13}C$ distribution in the F_2 population was near normal, with most of the plants occurring midway between the parents, even though the reciprocal F_1 hybrids differed by almost as much as the parents. This distribution of $\delta^{13}C$ was similar to that observed by Huber et al. (1989) for F_2 plants of *F. brownii* × *F. linearis* and Keefe (1987) for reciprocal backcrosses of *F. floridana* × *F. brownii* with *F. brownii*, except that in our larger population extreme segregates exceeded values of the parents (Fig. 1A). This is in contrast to $\delta^{13}C$ segregation in F_2 and F_3 populations of C_3 × C_4 *Atriplex* in which only values more negative than the F_1 and similar to the C_3 parent were obtained (Björkman et al., 1971). The greater and more normal segregation of $\delta^{13}C$ in our work and that of Huber et al. (1989) and Keefe (1987) may result from the closer phyletic relationship among *F. brownii*, *F. linearis*, and *F. floridana* (Powell, 1978) and more normal chromosome behavior in their F_1 hybrids (Powell, 1978; Huber et al., 1989) than was the case in *Atriplex*. *Flaveria brownii* is not completely C_4 (Cheng et al., 1989), and *F. linearis* is not completely C_3 (Monson et al., 1986); therefore, it is perhaps not surprising that hybrids between these species produce more fertile progeny and more segregation of traits than the C_3 × C_4 *Atriplex* hybrids. The divergence of $\delta^{13}C$ between *F. brownii* and *F. linearis*, however, is almost as great as in C_3 and C_4 species (7.4‰ in this paper, but 13.4‰ in the report by Byrd et al. [1992], 12.0‰ in the paper by Huber et al. [1989], and 10.5–11.5‰ in the report by Monson et al. [1988]). Differences between the C_4 species *F. trinervia* and C_3 *Flaveria* species range from 12.2 to 15.1‰ (Smith and Powell, 1984; Apel et al., 1988; Monson et al., 1988). Even though *F. brownii* and *F. linearis* appear closely related, the difference in degree of C_4 photosynthesis is large.

The continuous and more or less normal distribution of the F_2 progeny for $\delta^{13}C$, AP_{130}, and O_2 inhibition of *AP* (Fig. 1, A, B, and D, respectively) can be interpreted in several ways. Allard (1960) stated that these types of distributions for any trait among F_2 progeny can result from the action of either a few genes with moderately low heritability (i.e. large environmental influences) or a moderately large number of genes regardless of the heritability. These tests were not conducted in a manner that allowed us to obtain a classic estimate of environment and heritability (Allard, 1960); however, the

Table III. *Photosynthetic traits of selected F_2 plants from F. brownii × F. linearis*

Plant No.	AP_{130}[a]	Γ[a]	$\delta^{13}C$	^{14}C in C_4 Acids[a]
	μmol m^{-2} s^{-1}	μL L^{-1}	‰	%
87-612	13.4 ± 0.8	13 ± 1	−21.9	76 ± 6
87-559	12.5 ± 2.2	16 ± 3	−25.5	83 ± 2
87-567	12.1 ± 2.3	20 ± 3	−27.6	
87-614	9.5 ± 1.3	19 ± 2	−27.1	
87-639	9.5 ± 1.4	15 ± 1	−25.1	
87-641	9.1 ± 1.3	19 ± 4	−27.8	52 ± 3
Mean:	11.1 ± 1.9	17 ± 3	−25.8 ± 2.2	70 ± 16
87-569	8.8 ± 0.7	19 ± 3	−32.4	57 ± 16
87-608	8.4 ± 1.4	61 ± 4	−27.6	
87-570	8.3 ± 1.8		−34.2	48 ± 19
87-640	7.9 ± 1.1	18 ± 1	−27.2	
87-621	6.9 ± 1.8	19 ± 2	−27.4	
87-590	5.7 ± 0.9	18 ± 4	−28.4	55 ± 12
Mean:	7.7 ± 1.2	27 ± 19	−29.5 ± 3.0	53 ± 5

[a] Conditions for determination of these traits are the same as in Table I. Means ± SD are given for all traits, except $\delta^{13}C$, which represents only one determination. For group means the SD represents variation among F_2 progeny.

AP_{130} data were replicated over time, which did allow calculation of a general broad sense estimate of heritability (H). This estimate was high ($H = 0.84$) when using all F_2 progeny and means (at least within the constraints of this experiment) that the environmental influences were small and the trait is polygenic. If one assumes that the other traits would yield similar heritabilities, then our best speculation is that each measured trait is independently controlled by many genes. This speculation is consistent with the conclusion by Björkman (1976) that an efficient C_4 pathway requires that all the genes determining each component step in the pathway and their coordination must be inherited together.

Support for the idea of independent segregation of genes controlling various aspects of the C_4 cycle can be shown by production of plants very C_4 like in some traits and less C_4 like in others. The F_2 plant 87–612, most like *F. brownii* in $\delta^{13}C$ (−21.9‰), had O_2 inhibition of *AP* of 20.2 ± 1.6‰, a value much closer to *F. linearis* than *F. brownii*, and incorporated 76% of ^{14}C initially into malate and aspartate, compared with 92% in *F. brownii*. The reverse was also true, with the F_2 plant 87–569 having a $\delta^{13}C$ value slightly more negative than *F. linearis* but incorporating more than twice the percentage of ^{14}C into C_4 acids (57 versus 23‰, Tables I and III). Thus, correlation among the measured traits was low. This was also the case for presumably single-genes traits such as C_4 enzyme activities. For example, two F_2 plants (87–612 and 87–590) possessed in vitro NADP-ME activities that were equal to or approached that of the C_4-like parent, whereas PEPcase activities for these two plants more closely resembled the C_3-C_4 parent (Fig. 2).

In expression of C_4 traits, a change in genes controlling one component may be offset by genes controlling another component. For example, decreased O_2 sensitivity of *AP* may be accomplished by partitioning all of the Gly decarboxylase in BSC as occurs in C_3-C_4 species with no C_4 photosynthesis (Hylton et al., 1988) or by restricting all of the Rubisco in BSC and concentrating CO_2 by the C_4 cycle. In both instances, O_2 sensitivity of *AP* is probably reduced further by decreasing conductance of the BSC to CO_2. Therefore, decreased O_2 sensitivity that might be caused by increased activity of C_4 enzymes and even their proper compartmentation may be offset by greater permeability of BSC to CO_2. Genes controlling any aspect of these components would affect O_2 inhibition of *AP*, but complete O_2 insensitivity would require C_4 levels of enzyme activities, low conductance of BSC to CO_2, and presumably strict compartmentation of the enzymes.

Thus, understanding of inheritance of C_4 photosynthesis is complicated by the requisite metabolic interaction of the various components of the cycle. Results of this study have confirmed the independent segregation of component traits of the C_4 cycle observed earlier in $C_3 \times C_4$ *Atriplex* hybrids (Björkman, 1976). Also, it may be possible to transfer specific C_4 traits to non-C_4 species, at least when these species already have some C_4 tendencies as in certain C_3-C_4 intermediates. Whether C_4 photosynthesis can be transferred to C_3 species awaits further breeding to obtain fertile segregating progeny from $C_3 \times C_4$ hybrids.

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