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Photorespiration in C_3 and C_4 Plant Tissue Cultures

SIGNIFICANCE OF KRANZ ANATOMY TO LOW PHOTORESPIRATION IN C_4 PLANTS[1]

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ABSTRACT

Photorespiration rates in tissue cultures of a C_4 plant, *Portulaca oleracea*, were compared to those in tissue cultures of a C_3 plant, *Streptanthus tortuosus*. The C_4 plant tissue cultures have one-half to one-third the photorespiration rate of the C_3 plant tissue cultures and respond to varying O_2 concentrations in a manner typical of C_4 plants. The results suggest that the lack of detectable photorespiration in C_4 plants is not related to leaf anatomy.

Photorespiration is of great interest because of its relationship to plant productivity. It has been estimated that in C_3 plants, the process of photorespiration oxidizes up to 50% of the newly synthesized photosynthetic products, whereas in C_4 plants, photorespiration is either not present or occurs in much reduced amounts (20). In addition to the lack of apparent photorespiration and significantly greater net assimilation rate, C_4 plants also possess several other structural and physiological features which distinguish them from C_3 plants. These include: a different chloroplast ultrastructure, reduced discrimination of ^{13}C relative to ^{12}C, higher temperature optimum for photosynthesis, higher light saturation point, and significantly lower transpiration to photosynthesis ratios (13, 19).

One of the most interesting features of C_4 plants is that virtually all of them have a characteristic leaf anatomy consisting of at least two chloroplast-containing cell layers radially arranged around the vascular bundles. This concentric arrangement of chlorenchyma cells in C_4 plant leaves has been called "Kranz" anatomy (13), and many structure-function postulations have been made concerning the importance of Kranz anatomy to the over-all physiology of C_4 plants. There is general agreement that operation of the complete cycle of C_4 reactions is closely associated with, if not dependent upon, possession of Kranz anatomy by C_4 plants. This is particularly true for the apparent lack of photorespiration. As envisioned, the concentric arrangement of chloroplast-containing cells in C_4 plants acts either to inhibit photorespiratory CO_2 loss by high CO_2 concentrations in the bundle sheath cells, or to promote refixation of evolved CO_2 as it diffuses outwardly through the mesophyll cells. A discussion and models for such structure-function relationships in C_4 plants have recently been given (13).

Regardless of the exact mechanism, most reports agree on the singular importance of Kranz anatomy in minimizing or eliminating detectable CO_2 evolution in the light by C_4 plants (1, 3, 7, 15, 16). Recently, several "atypical" features of C_4 plant physiology have been published where strict structure-function relationships do not hold. In spite of an intact Kranz anatomy, it has been shown that there is an increased operation of the C_3 cycle during certain stages of leaf development in some C_4 plants (10, 12), a substantial incorporation of $^{14}CO_2$ into both four-carbon acids and phosphorylated compounds in a plant with an intermediate C_4 anatomy (11), and C_4 acid production in C_4 plant tissue cultures (14). There are also studies which cast doubt on the essentiality of Kranz anatomy to C_4 physiology with respect to photorespiration. Goldsworthy and Day (6) have questioned the importance of Kranz anatomy in restricting diffusionary loss of CO_2 by C_4 plants. Other studies have reported lowered photorespiration in a plant with an incomplete Kranz anatomy (10), and photorespiratory responses in senescing *Portulaca oleracea* leaves which are virtually indistinguishable from those of mature C_3 plant leaves (9). In spite of these examples, operation of the C_4 pathway, particularly the lack of detectable photorespiration in C_4 plants, is still thought by many to be dependent on an intact Kranz anatomy.

MATERIALS AND METHODS

In the present experiments, *P. oleracea* L. stem tissue cultures, grown under a controlled light (about 80 μeinsteins m^{-2} sec^{-1}) and temperature regime (20 C) were used in all experiments. Cultures, conditions, and media were otherwise as reported by Laetsch and Kortschak (14). These tissues were similar to mature *P. oleracea* leaves on the basis of cell ultrastructure and $^{14}CO_2$ labeling patterns (10, unpublished observations). In addition, leaves and tissue cultures of a C_3 plant, *Streptanthus tortuosus*, were used to enable comparison of photorespiratory activity in C_3 plant tissues to those of C_4 plants. Photorespiration assays used were modified from Zelitch (18) and involve a determination of the $^{14}CO_2$ evolved in the light and dark, after a 45-min $^{14}CO_2$ assimilation period (11).

Experiments were conducted in 125- or 250-ml Erlenmeyer flasks containing 3 to 5 g of callus tissue, and in some experiments, 50 ml of culture medium. No difference was observed between experiments in which the tissue cultures remained on the nutrient medium or were placed in similar flasks containing a thin layer of water. After a 30-min equilibration period, 0.3 μmol $^{14}CO_2$ was injected into sealed flasks for assimilation. Radioactive CO_2 given off in 30-min light and dark periods was then determined by passing CO_2-free air through the flask at 600 ml min^{-1}. The first 15 min of $^{14}CO_2$ evolution in both the light and dark phase was generally not used for counting, and the order in which the light and dark periods were used was unimportant. All experiments were conducted in a water bath maintained at 30 C with a light intensity of 800 to 1000 μeinsteins m^{-2} sec^{-1} at the callus surface. $^{14}CO_2$ evolved was trapped in 1 N KOH with 6% isoamyl alcohol.

RESULTS

Table I characterizes Chl content, $^{14}CO_2$ assimilation and evolution data for *Portulaca* and *Streptanthus* leaves and tissue

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Table I. *Chlorophyll, $^{14}CO_2$ Assimilated, and $^{14}CO_2$ Evolved for C_4 (P. oleracea) and C_3 (S. tortuosus) Plant Tissue Cultures and Whole Leaves*

	Chlorophyll		$^{14}CO_2$ assimilated				$^{14}CO_2$ evolved			
			rate		cpm x 10^{-3}		light		dark	
	mg / g f wt	a/b ratio	μmole / g f wt / hr	μmole / mg chl/hr	g f wt / hr	mg chl / hr	cpm x 10^{-3} / g f wt	cpm x 10^{-3} / mg chl	cpm x 10^{-3} / g f wt	cpm x 10^{-3} / mg chl
Young Portulaca leaves	0.91	3.2	10.19	11.20	3280	3606	1886	2072	1820	2000
Portulaca callus	0.12	2.0	1.35	11.26	446	3626	58	471	64	520
Young Streptanthus leaves	1.93	2.1	--	--	--	--	5663	2934	2056	1065
Streptanthus callus	0.10	2.4	1.23	12.2	406	3942	286	2776	94	913

[1] 30 min. evolution period in CO_2-free air.

cultures. Both tissue cultures contain nearly identical amounts of Chl and have similar Chl *a/b* ratios. The Chl *a/b* ratio for the C_4 callus is, understandably, not as high as that of whole *Portulaca* leaves, nor as high as is generally found in C_4 plant leaves (2, 8). Assimilation rates for both tissue cultures are quite high. When expressed on a Chl basis, the photosynthetic rate of *Portulaca* tissue cultures was slightly greater than that of young *Portulaca* leaves.

The difference in photorespiration between C_3 and C_4 tissues can be noticed by comparing the $^{14}CO_2$ evolved in the light to that in the dark. Unlike C_3 plants, there is no increased light-dependent $^{14}CO_2$ evolution in C_4 plants (18). It is important to note in Table I that the major difference between C_3 and C_4 tissue cultures, as in whole leaves, is in the amount of $^{14}CO_2$ given off in the light compared to that in the dark. This basic difference between C_3 and C_4 plant tissues is easily seen by examining light to dark ratios of CO_2 evolution in CO_2-free air (Table II). As in intact leaves (9), *Portulaca* tissue cultures have light to dark ratios of approximately 1, whereas *Streptanthus* tissue cultures have light to dark ratios of nearly 3. Such values are characteristic of C_4 and C_3 plants, respectively (9, 11, 18). Both types of callus fix about the same amount of ^{14}C in the light and evolve similar amounts in the dark.

Another method of illustrating the photorespiratory differences between C_3 and C_4 plant tissues is to determine their response to varying O_2 concentrations (9). C_4 plant tissues evolve relatively constant amounts of CO_2, regardless of the gas treatment, whereas C_3 plants are very much affected by O_2 concentration (4, 5, 9, 18). For *Portulaca* tissue cultures, a N_2 atmosphere does not markedly decrease photorespiration, nor does O_2 gassing increase it (Table II). *Streptanthus* tissue cultures, on the other hand, exhibit O_2 responses typical of C_3 tissues. These responses can best be seen in Figure 1. Under 21% O_2 ($-CO_2$), and especially in 100% O_2, *Streptanthus* tissue cultures had light CO_2 evolution rates significantly above those of the C_4 callus (Fig. 1, left, right). When exposed to a N_2 atmosphere, however, the C_3 callus exhibited photorespiratory responses virtually indistinguishable from those of *Portulaca* (Fig. 1, center). The extent of the differences between C_3 and C_4 plant tissue cultures can be seen by examining the slopes in Figure 1. Whereas the $^{14}CO_2$ evolution rates for the C_3 callus are nearly twice those of the C_4 callus in CO_2-free air, the C_3 rate is over 3 times greater under 100% O_2. However, under conditions which virtually eliminate photorespiration in C_3 plants (N_2 atmosphere, Fig. 1, center), the rate and slope of the C_3 tissue culture are decreased to the level of the C_4 callus. Also, since the slopes for CO_2 evolution in the dark are nearly identical, it is the photorespiratory CO_2 release which is the truly distinguishing feature.

Table II. *Effect of $-CO_2$, N_2, and O_2 on Photorespiratory $^{14}CO_2$ Released by C_4 (P. orleracea) and C_3 (S. tortuosus) Tissue Cultures*

Expt. No.	Plant Tissue	Gas phase	$^{14}CO_2$ released/ g f wt light	$^{14}CO_2$ released/ g f wt dark	Light / dark ratio[1]
			cpm x 10^{-3}		
1	Portulaca callus	$-CO_2$	58	64	0.91
2	Portulaca callus	N_2	40	25	--
3	Portulaca callus	O_2	54	43	--
4	Streptanthus callus	$-CO_2$	111	42	2.64
5	Streptanthus callus	N_2	61	15	--
6	Streptanthus callus	O_2	224	80	--
7	Portulaca leaves	$-CO_2$	1080	1003	1.08
8	Streptanthus leaves	$-CO_2$	5663	2056	2.75

[1] Averages of at least 3 separate experiments.

DISCUSSION

The unique physiological and biochemical features of C_4 plants have generally been considered to be causally linked to their characteristic Kranz anatomy. The present data suggest that such a one-to-one correspondence does not exist. This is also supported by previous work. Laetsch and Kortschak (14) showed that four carbon acids were the most heavily labeled compounds produced by tissue cultures of another C_4 plant and concluded "that carbon fixation pathways are not related to leaf and chloroplast structure." Similar results have been obtained with *P. oleracea* tissue cultures. In short term $^{14}CO_2$ assimilation experiments, the early labeled products of *Portulaca* tissue cultures are similar to those of whole leaves. The ^{14}C content in C_4 acids decreases with time in pulse-chase experiments, while that in C_3 cycle products increases (unpublished results). The role of specialized leaf anatomy in C_4 plants was also questioned when a plant with incomplete Kranz anatomy, but substantial labeling of

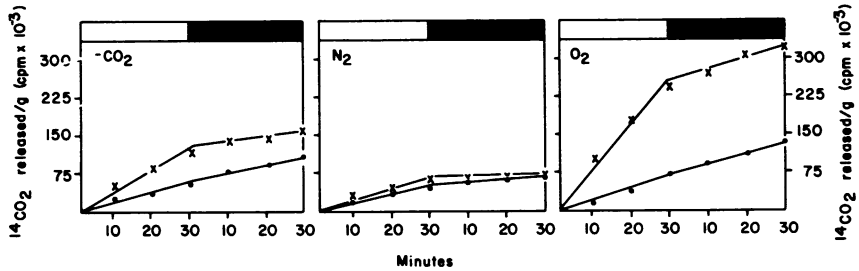

FIG. 1. $^{14}CO_2$ evolved in the light and dark for *Portulaca oleracea* (●——●) and *Streptanthus tortuosus* (×——×) tissue cultures. The tissue cultures were allowed to assimilate completely 0.3 μmol $^{14}CO_2$ for 45 min at 30 C and 800 to 1000 μeinsteins m^{-2} sec^{-1}. Subsequently, CO_2-free air (21% O_2), N_2, or 100% O_2 was passed over the tissues and the $^{14}CO_2$ evolved in the light was compared to that given off in the dark.

four carbon acids, still exhibited much reduced photorespiration ratios (11). Shomer-Ilan and Waisel (17) have also suggested modification of the Kranz requirement for operation of the C_4 pathway based on typical C_4 pathway carbon reactions in a plant without Kranz.

These examples illustrate that plants with functional C_4 physiology may also possess Kranz anatomy, but are not dependent on it. C_4 anatomy may continue to be very important in the taxonomy of C_4 plants, and it may explain their increased water-use efficiency and ability to grow in habitats of limited water. It does not appear that Kranz anatomy is essential to proper functioning of carbon metabolism in C_4 plants, including low photorespiration. Also, the present data do not agree with models that propose that operation of the C_4 pathway and/or lack of photorespiration is dependent on a particular anatomy. If an inhibition of photorespiration by high CO_2 concentrations occurs in C_4 plants, it may happen within the bundle sheath chloroplasts as suggested much earlier (6), but not within the bundle sheath cells as a whole.

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