Photorespiration in C₃ and C₄ Plant Tissue Cultures

SIGNIFICANCE OF KRANZ ANATOMY TO LOW PHOTORESPIRATION IN C₄ PLANTS

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ABSTRACT

Photorespiration rates in tissue cultures of a C₃ plant, Portulaca oleracea, were compared to those in tissue cultures of a C₄ plant, Streptanthus tortuosus. The C₃ plant tissue cultures have one-half to one-third the photorespiration rate of the C₄ plant tissue cultures and respond to varying O₂ concentrations in a manner typical of C₃ plants. The results suggest that the lack of detectable photorespiration in C₄ 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 C₃ plants, the process of photorespiration oxidizes up to 50% of the newly synthesized photosynthetic products, whereas in C₄ 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, C₄ plants also possess several other structural and physiological features which distinguish them from C₃ plants. These include: a different chloroplast ultrastructure, reduced discrimination of ¹³C relative to ¹²C, 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 C₄ 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 C₄ 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 C₄ plants. There is general agreement that operation of the complete cycle of C₄ reactions is closely associated with, if not dependent upon, possession of Kranz anatomy by C₄ plants. This is particularly true for the apparent lack of photorespiration. As envisioned, the concentric arrangement of chloroplast-containing cells in C₄ plants acts either to inhibit photorespiratory CO₂ loss by high CO₂ concentrations in the bundle sheath cells, or to promote re-fixation of evolved CO₂ as it diffuses outwardly through the mesophyll cells. A discussion and models for such structure-function relationships in C₄ 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 CO₂ evolution in the light by C₄ plants (1, 3, 7, 15, 16). Recently, several "atypical" features of C₄ 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 C₃ cycle during certain stages of leaf development in some C₄ plants (10, 12), a substantial incorporation of ¹⁴CO₂ into both four-carbon acids and phosphorylated compounds in a plant with an intermediate C₄ anatomy (11), and C₄ acid production in C₄ plant tissue cultures (14). There are also studies which cast doubt on the essentiality of Kranz anatomy to C₄ physiology with respect to photorespiration. Goldsworthy and Day (6) have questioned the importance of Kranz anatomy in restricting diffusional loss of CO₂ by C₄ plants. Other studies have reported lowered photorespiration in a plant with an incomplete Kranz anatomy (10), and photorespiratory responses in seasing Portulaca oleracea leaves which are virtually indistinguishable from those of mature C₃ plant leaves (9). In spite of these examples, operation of the C₄ pathway, particularly the lack of detectable photorespiration in C₄ 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 ¹⁴CO₂ labeling patterns (10, unpublished observations). In addition, leaves and tissue cultures of a C₃ plant, Streptanthus tortuosus, were used to enable comparison of photorespiratory activity in C₄ plant tissues to those of C₃ plants. Photorespiration assays used were modified from Zelitch (18) and involve a determination of the ¹⁴CO₂ evolved in the light and dark, after a 45-min ¹⁴CO₂ assimilation period (11).

Experiments were conducted in 125- or 250-ml Erlemeyer 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 ¹⁴CO₂ was injected into sealed flasks for assimilation. Radioactive CO₂ given off in 30-min light and dark periods was then determined by passing CO₂-free air through the flask at 600 ml min⁻¹. The first 15 min of ¹⁴CO₂ 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. ¹⁴CO₂ evolved was trapped in 1 N KOH with 6% isoamyl alcohol.

RESULTS

Table I characterizes Chl content, ¹⁴CO₂ assimilation and evolution data for Portulaca and Streptanthus leaves and tissue.
cultures. Both tissue cultures contain nearly identical amounts of Chl and have similar Chl a/b ratios. The Chl a/b ratio for the C₄ callus is, understandably, not as high as that of whole Portulaca leaves, nor as high as is generally found in C₃ 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 C₃ and C₄ tissues can be noticed by comparing the ¹⁴CO₂ evolved in the light to that in the dark. Unlike C₃ plants, there is no increased light-dependent ¹⁴CO₂ evolution in C₄ plants (18). It is important to note in Table I that the major difference between C₃ and C₄ tissue cultures, as in whole leaves, is in the amount of ¹⁴CO₂ given off in the light compared to that in the dark. This basic difference between C₃ and C₄ plant tissues is easily seen by examining light to dark ratios of ¹⁴CO₂ evolution in CO₂-free air (Table II). 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 C₄ and C₃ plants, respectively (9, 11, 18). Both types of callus fix about the same amount of ¹⁴C in the light and evolve similar amounts in the dark.

Another method of illustrating the photorespiratory differences between C₃ and C₄ plant tissues is to determine their response to varying O₂ concentrations (9). C₃ plant tissues evolve relatively constant amounts of CO₂, regardless of the gas treatment, whereas C₄ plants are very much affected by O₂ concentration (4, 5, 9, 18). For Portulaca tissue cultures, a N₂ atmosphere does not markedly decrease photorespiration, nor does O₂ gassing increase it (Table II). Streptanthus tissue cultures, on the other hand, exhibit O₂ responses typical of C₃ tissues. These responses can best be seen in Figure 1. Under 21% O₂ (−CO₂), and especially in 100% O₂, Streptanthus tissue cultures had light CO₂ evolution rates significantly above those of the C₄ callus (Fig. 1, left, right). When exposed to a N₂ atmosphere, however, the C₄ callus exhibited photorespiratory responses virtually indistinguishable from those of Portulaca (Fig. 1, center). The extent of the differences between C₃ and C₄ plant tissue cultures can be seen by examining the slopes in Figure 1. Whereas the ¹⁴CO₂ evolution rates for the C₄ callus are nearly twice those of the C₄ callus in CO₂-free air, the C₃ rate is over 3 times greater under 100% O₂. However, under conditions which virtually eliminate photorespiration in C₃ plants (N₂ atmosphere, Fig. 1, center), the rate and slope of the C₄ tissue culture are decreased to the level of the C₃ callus. Also, since the slopes for CO₂ evolution in the dark are nearly identical, it is the photorespiratory CO₂ release which is the truly distinguishing feature.

DISCUSSION

The unique physiological and biochemical features of C₄ 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 C₄ 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 ¹⁴CO₂ assimilation experiments, the early labeled products of Portulaca tissue cultures are similar to those of whole leaves. The ¹⁴C content in C₄ acids decreases with time in pulse-chase experiments, while that in C₃ cycle products increases (unpublished results). The role of specialized leaf anatomy in C₄ plants was also questioned when a plant with incomplete Kranz anatomy, but substantial labeling of

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**Table I. Chlorophyll, ¹⁴CO₂ Assimilated, and ¹⁴CO₂ Evolved for C₄ (P. oleracea) and C₃ (S. tortuosus) Plant Tissue Cultures and Whole Leaves**

<table>
<thead>
<tr>
<th>Plant Tissue</th>
<th>Chl mg/g chl/hr</th>
<th>¹⁴CO₂ evolved g f wt/hr</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Portulaca leaves</td>
<td>0.91</td>
<td>10.19</td>
<td>11.20</td>
<td>3280</td>
</tr>
<tr>
<td>Portulaca callus</td>
<td>0.12</td>
<td>20</td>
<td>11.26</td>
<td>446</td>
</tr>
<tr>
<td>Young Streptanthus leaves</td>
<td>1.93</td>
<td>21</td>
<td>17</td>
<td>563</td>
</tr>
<tr>
<td>Streptanthus callus</td>
<td>0.10</td>
<td>2.41</td>
<td>2.12</td>
<td>406</td>
</tr>
</tbody>
</table>

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**Table II. Effect of −CO₂, N₂, and O₂ on Photorespiratory ¹⁴CO₂ Released by C₄ (P. oleracea) and C₃ (S. tortuosus) Tissue Cultures**

<table>
<thead>
<tr>
<th>Expt. Plant Tissue</th>
<th>Gas phase g f wt</th>
<th>¹⁴CO₂ released Light dark</th>
<th>g f wt/hr</th>
<th>Light dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Portulaca callus</td>
<td>−CO₂</td>
<td>58</td>
<td>64</td>
<td>0.91</td>
</tr>
<tr>
<td>2 Portulaca callus</td>
<td>N₂</td>
<td>40</td>
<td>25</td>
<td>--</td>
</tr>
<tr>
<td>3 Portulaca callus</td>
<td>O₂</td>
<td>54</td>
<td>45</td>
<td>--</td>
</tr>
<tr>
<td>4 Streptanthus callus</td>
<td>−CO₂</td>
<td>111</td>
<td>42</td>
<td>2.64</td>
</tr>
<tr>
<td>5 Streptanthus callus</td>
<td>N₂</td>
<td>115</td>
<td>55</td>
<td>--</td>
</tr>
<tr>
<td>6 Streptanthus callus</td>
<td>O₂</td>
<td>224</td>
<td>80</td>
<td>--</td>
</tr>
<tr>
<td>7 Portulaca leaves</td>
<td>−CO₂</td>
<td>1060</td>
<td>1003</td>
<td>1.28</td>
</tr>
<tr>
<td>8 Streptanthus leaves</td>
<td>−CO₂</td>
<td>5663</td>
<td>2056</td>
<td>2.75</td>
</tr>
</tbody>
</table>

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**Fig. 1.** ¹⁴CO₂ 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 ¹⁴CO₂ for 45 min at 30°C and 800 to 1000 μEinsteins m⁻² s⁻¹. Subsequently, CO₂-free air (21% O₂), N₂, or 100% O₂ was passed over the tissues and the ¹⁴CO₂ 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 C₄ pathway based on typical C₄ pathway carbon reactions in a plant without Kranz.

These examples illustrate that plants with functional C₄ physiology may also possess Kranz anatomy, but are not dependent on it. C₄ anatomy may continue to be very important in the taxonomy of C₄ 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 C₄ plants, including low photorespiration. Also, the present data do not agree with models that propose that operation of the C₄ pathway and/or lack of photorespiration is dependent on a particular anatomy. If an inhibition of photorespiration by high CO₂ concentrations occurs in C₄ 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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LITERATURE CITED