

A COMPARATIVE STUDY OF THE EPIDERMIS, MESOPHYLL AND WHOLE LEAF OF C₃ AND CAM PLANTS ON THEIR CARBON ISOTOPE RATIOS

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ABSTRACT

Carbon isotope ratios of the epidermis, mesophyll and whole leaf tissues of plants belonging to both C₃ and CAM photosynthetic categories were compared. $\delta^{13}\text{C}$ values for the epidermis were significantly different (at $P < 0.005$ level) and more negative from those of both mesophyll and whole leaf tissues. The findings of this study are consistent with the view that guard cell photosynthesis does not contribute substantially to the epidermal biomass and the carbon in epidermal tissues is apparently fixed in the mesophyll and transported to the surface.

INTRODUCTION

DIFFERENCES in $^{13}\text{C}/^{12}\text{C}$ ratios of plant tissues indicate source carbon¹ and distinguish different photosynthetic categories of plants²⁻⁴. Isotopic discrimination of carbon is due chiefly to discrimination by the initial carboxylating enzymes, namely RuBP carboxylase in C₃ plants⁵ and PEP carboxylase in C₄ plants⁶.

The major pathway of CO₂ fixation in guard cells of many plant species involves PEP carboxylase⁷⁻¹⁰. The occurrence of RuBP carboxylase in the guard cells has been demonstrated in some CAM plants¹¹, however most plants display an almost negligible activity of this enzyme¹². Willmer and Firth¹³ found that the epidermis was slightly less negative than that of mesophyll tissues in their $\delta^{13}\text{C}$ values. Nishida *et al*¹⁴ found that $\delta^{13}\text{C}$ values of the epidermis of *Kalanchoe daigremontiana* (a CAM plant) were strikingly more negative than those of mesophyll tissues. The present study was undertaken to determine the differences, if any, in the carbon isotopic ratios of epidermal and mesophyll tissues of representatives from both C₃ and CAM plants.

MATERIALS AND METHODS

Sedum pachyphyllum Rose, *Kalanchoe holstii* Engl., *Senecio serpens* Rowl., *Plectranthes aus-*

tralis L., Her., *Crassula arborescens* (Mill) Willd., and *Senecio mikanoides* Otto. were all grown in the greenhouse. The epidermis, mesophyll (without the lower epidermis in all plants except *Sedum pachyphyllum*) and whole leaf (modified stems in some cases) samples of all plants listed above were collected and oven dried at 80°C for 48 hr. Epidermal strips were obtained from fully expanded mature leaves of all species and were carefully screened for freedom from mesophyll contamination. Each sample was totally combusted in a stream of oxygen at 800°C over copper turnings. Combustion products were trapped in liquid nitrogen and the excess oxygen pumped away. The trap was then warmed to dry ice temperature, which retained water as ice and set free the CO₂ for collection in a sample tube. Samples of CO₂ thus collected were analysed for $^{13}\text{C}/^{12}\text{C}$ on an isotope ratio mass spectrometer, with a precision of 0.09‰ for each determination. The $^{13}\text{C}/^{12}\text{C}$ ratio is expressed as $\delta^{13}\text{C}$ with reference to the PDB standard (Pee Dee Belemnite from South Carolina).

Following the method of Willmer and Firth¹³ compounds were extracted from the epidermal and mesophyll tissues of *Sedum pachyphyllum* (a CAM species) in hot 80% (v/v) ethanol, followed by hot 50% (v/v) ethanol and then water. The ethanol/water soluble and insoluble fractions were pooled for each tissue. The fractions were

then dried, ground to a powder, totally combusted and analysed on the mass spectrometer.

RESULTS AND DISCUSSION

The $\delta^{13}\text{C}$ values for epidermal tissues in all species analysed in this study, were consistently more negative than the mesophyll tissues (table 1) and this is in accordance with the findings of Nishida *et al*¹⁴. The $\delta^{13}\text{C}$ values for the whole leaf tissues were less negative than those of the epidermal tissues (table 1). Differences in the carbon isotopic ratios between the epidermal tissues and whole leaf or mesophyll tissues, when compared in a paired *t*-test were significant at $P < 0.005$ level whereas the differences in $\delta^{13}\text{C}$ values between whole leaf and mesophyll tissues were found to be non-significant. The $\delta^{13}\text{C}$ values for the soluble (ethanol/water) fractions were consistently more

negative than the insoluble fractions, in both epidermal and mesophyll tissues of *Sedum* (table 2). These results agree with those of Willmer and Firth¹³.

Our findings are consistent with the view that guard cell photosynthesis does not contribute substantially to the epidermal biomass. Carbon in epidermal tissues is apparently fixed in the mesophyll and transported to the surface. More negative values for epidermal tissues and the ethanol-soluble fraction may simply reflect the influence of cuticular and other lipids¹⁵.

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Table 1 ^{13}C (‰) vs. PDB values for the epidermal and mesophyll tissues of C_3 and CAM species. The values listed below represent the mean value of at least three replications.

Species	Photo-synthetic pathway type			
		Epidermis	Mesophyll*	Leaf
<i>Sedum</i>				
<i>pachyphyllum</i> **	CAM	-18.2	-14.4	-15.0
<i>Senecio serpens</i>	CAM	-18.8	-15.7	-16.2
<i>Crassula</i>				
<i>arborescens</i>	CAM	-16.8	-13.1	-15.0
<i>Kalanchoe holstii</i>	C_3	-24.6	-21.4	-22.3
<i>Plectranthes</i>				
<i>australis</i>	C_3	-26.7	-23.8	-24.7
<i>Senecio mikanoides</i>	C_3	-26.3	-23.4	-26.7

* Mesophyll had the upper epidermis attached

** In all species except *Sedum* the lower epidermis alone was analysed

Table 2 ^{13}C (‰) vs PDB values for the soluble and insoluble (in ethanol/water) fractions of epidermal and mesophyll tissues of *Sedum pachyphyllum*. All values are mean of three independent analyses of each tissue.

Fractions	Epidermis	Mesophyll
Soluble	-20.6	-17.6
Insoluble	-15.4	-13.1

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