Introduction

Climate warming and atmospheric CO₂ enrichment provide a competitive advantage to plants that do not invest resources in a carbon concentrating mechanism (CCM) to reduce photosynthetic inhibition [1].

Crassulacean acid metabolism (CAM) plants can deactivate their CCMs under high CO₂ concentrations, reducing their fitness in arid environments relative to C₃ photosynthetic plants [1].

CAM plants’ response to global change can be evaluated through comparative analysis of CCM strength via carbon isotope ratios (δ¹³C) of photosynthetic tissue [2]. Starch pool δ¹³C include only recently assimilated carbon and thus more accurately represent CAM activity than bulk tissue δ¹³C, especially in intermediate C₃+CAM plants [2].

We assess the efficacy of starch extraction for δ¹³C analysis through study of a C₃ plant, an obligate CAM plant, and an intermediate C₃+CAM plant.

Methods

Leaves of the C₃ plant Alternanthera sessilis, leaves of the obligate CAM plant Kalanchoë daigremontiana, and leaves and stems of the C₃+CAM plant Bulnesia retama were sampled in the late afternoon of sunny days [2,3,4]. Samples were dried then treated with methanol and chloroform to isolate soluble starch [2]. Starch was boiled in solution with diH₂O until gelatinized, then treated with α-amylase to convert the starch to sugars [2]. δ¹³C of the isolated sugars and of dried bulk tissue from all three species were determined at the Washington State University Stable Isotope Core Laboratory.

Results

- Starch δ¹³C are significantly less negative than bulk tissue δ¹³C in Bulnesia retama (C₃+CAM) leaves and stems.
- There is no significant difference between starch and bulk tissue δ¹³C in the leaves of Alternanthera sessilis (C₃) and Kalanchoë daigremontiana (CAM).

Discussion

CCMs discriminate less against ¹³CO₂ than the C₃ cycle, making δ¹³C useful in differentiating between C₃ and CAM plants [4].

- Strong CAM δ¹³C range: [-10‰, -20‰]
- C₃+CAM δ¹³C range: [-20‰, -25‰]
- C₃ δ¹³C range: [-21‰, -32‰]

The δ¹³C difference between bulk leaf and starch samples in Bulnesia retama (C₃+CAM) is due to CCM activity in addition to exclusion of post-fixation isotope discrimination in the starch pool [4].

The small difference between bulk leaf and starch δ¹³C in Kalanchoë daigremontiana (CAM) can be attributed in part to C₃ cycle activity in CAM photosynthesis phases II and IV [1,unpublished data].

Starch extraction for δ¹³C analysis is a more accurate method for assessing relative CAM strength in C₃+CAM plants than use of bulk tissue, which will be useful in assessing both CAM loss under elevated atmospheric CO₂ and CAM evolutionary intermediacy.

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References