

C₄ photosynthesis in terrestrial plants does not require Kranz anatomy

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C₄ photosynthesis in terrestrial plants was thought to require Kranz anatomy because the cell wall between mesophyll and bundle sheath cells restricts leakage of CO₂. Recent work with the central Asian chenopods *Borszczowia aralocaspica* and *Bienertia cycloptera* show that C₄ photosynthesis functions efficiently in individual cells containing both the C₄ and C₃ cycles. These discoveries provide new inspiration for efforts to convert C₃ crops into C₄ plants because the anatomical changes required for C₄ photosynthesis might be less stringent than previously thought.

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C₄ photosynthesis is the major carbon-concentrating mechanism used by land plants to compensate for limitations associated with low atmospheric CO₂ [1]. C₄ plants concentrate CO₂ by first carboxylating phosphoenolpyruvate (PEP) in an outer layer of photosynthetic mesophyll cells [termed the photosynthetic carbon assimilation (PCA) cells; Fig. 1]. The resulting four-carbon acids (C₄ acids) diffuse into the bundle sheath layer of cells where ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the photosynthetic carbon reduction (PCR) reactions are localized. Together, the two cell layers confer a wreath-like appearance upon the leaf anatomy, which is termed Kranz anatomy [2]. In the PCR cells, the C₄ acids are decarboxylated to produce CO₂ and a three-carbon organic acid. The C₃ acid returns to the PCA layer to be converted to PEP while the released CO₂ accumulates around Rubisco at concentrations high enough to suppress photorespiration.

Although C₄ photosynthesis is often considered to be a single biochemical pathway, it is actually accomplished by more than a dozen biochemical and anatomical combinations that arose independently in the past 30 million years [1,3]. In spite of these variations, all C₄ plants share the common initial step of

PEP carboxylation, and, until recently, it was thought that Kranz anatomy was also essential for C₄ photosynthesis in terrestrial plants [3]. Three new studies challenge the view that Kranz anatomy is a requirement for C₄ photosynthesis by demonstrating that C₄ photosynthesis functions in single cells of the central Asian chenopods, *Borszczowia aralocaspica* and *Bienertia cycloptera* [4–6].

Significance of Kranz anatomy

An essential feature of any carbon-concentrating mechanism is the ability to restrict gas diffusion out of the high CO₂ compartment. In aquatic algae and some aquatic plants, carbon-concentrating mechanisms occur in single cells, but leakage is less of a concern because the surrounding aqueous matrix restricts

diffusion out of the cell. Most algae concentrate inorganic carbon [7]; however, some aquatic angiosperms (*Hydrilla verticillata* and *Egeria densa*) and diatoms (*Thalassiosira weissflogii*) are reported to operate a complete C₄ photosynthetic cycle in individual photosynthetic cells [8–10]. In contrast to the situation in aquatic habitats, there is no surrounding water to restrict efflux of concentrated CO₂ in terrestrial plants. Thus, it was thought that a modified wall separating PCA and PCR cells was essential for the efficient function of C₄ photosynthesis in terrestrial plants. In particular, the wall is highly resistant to CO₂ loss while allowing rapid metabolite diffusion between PCA and PCR cells via an extensive network of plasmodesmata [2,11]. Given these considerations, and

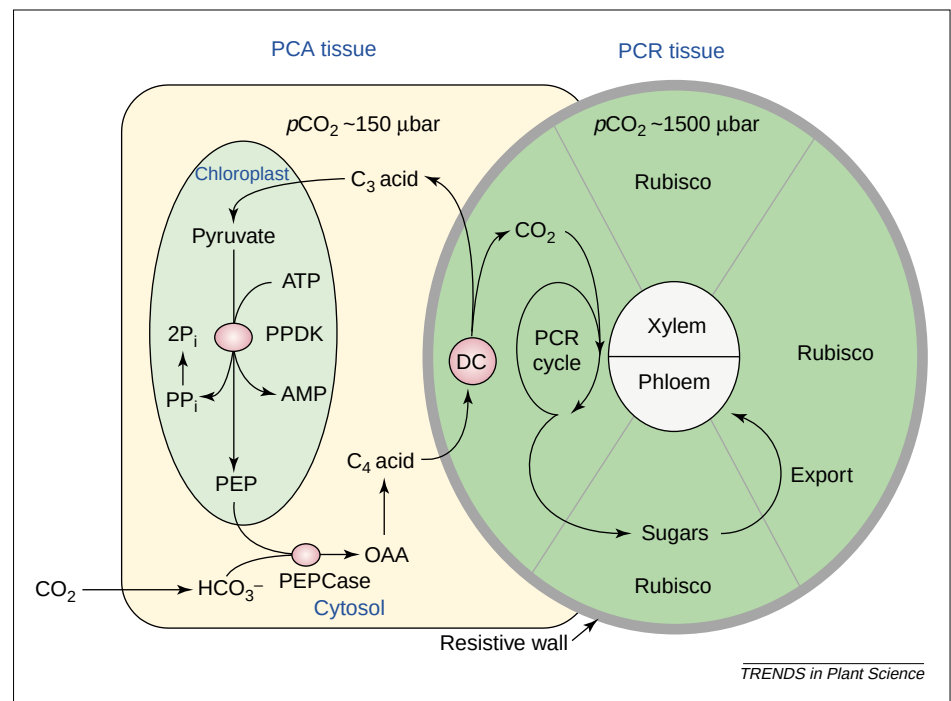


Fig. 1. C₄ photosynthesis. In the photosynthetic carbon assimilation (PCA) tissue (also termed mesophyll tissue), the carboxylation of phosphoenolpyruvate (PEP) by PEP carboxylase (PEPCase) occurs to form oxaloacetic acid (OAA), which is then converted to another C₄ acid (malate or aspartate). The C₄ acid diffuses via plasmodesmata to the bundle sheath tissue where Rubisco and the photosynthetic carbon reduction (PCR) cycle is located. A decarboxylase enzyme (DC) decarboxylates the C₄ acid in the bundle sheath cells and the CO₂ concentration rises to a partial pressure (pCO₂) that is ~10 times that in mesophyll cells. The C₃ acid released by the decarboxylation step returns to the mesophyll chloroplasts where it is phosphorylated by pyruvate, phosphate dikinase (PPDK) to produce PEP, thus completing the C₄ cycle. Rubisco is localized in the bundle sheath cells, where it carboxylates CO₂ in the first step of the PCR cycle. Abbreviation: PP_i - pyrophosphate.

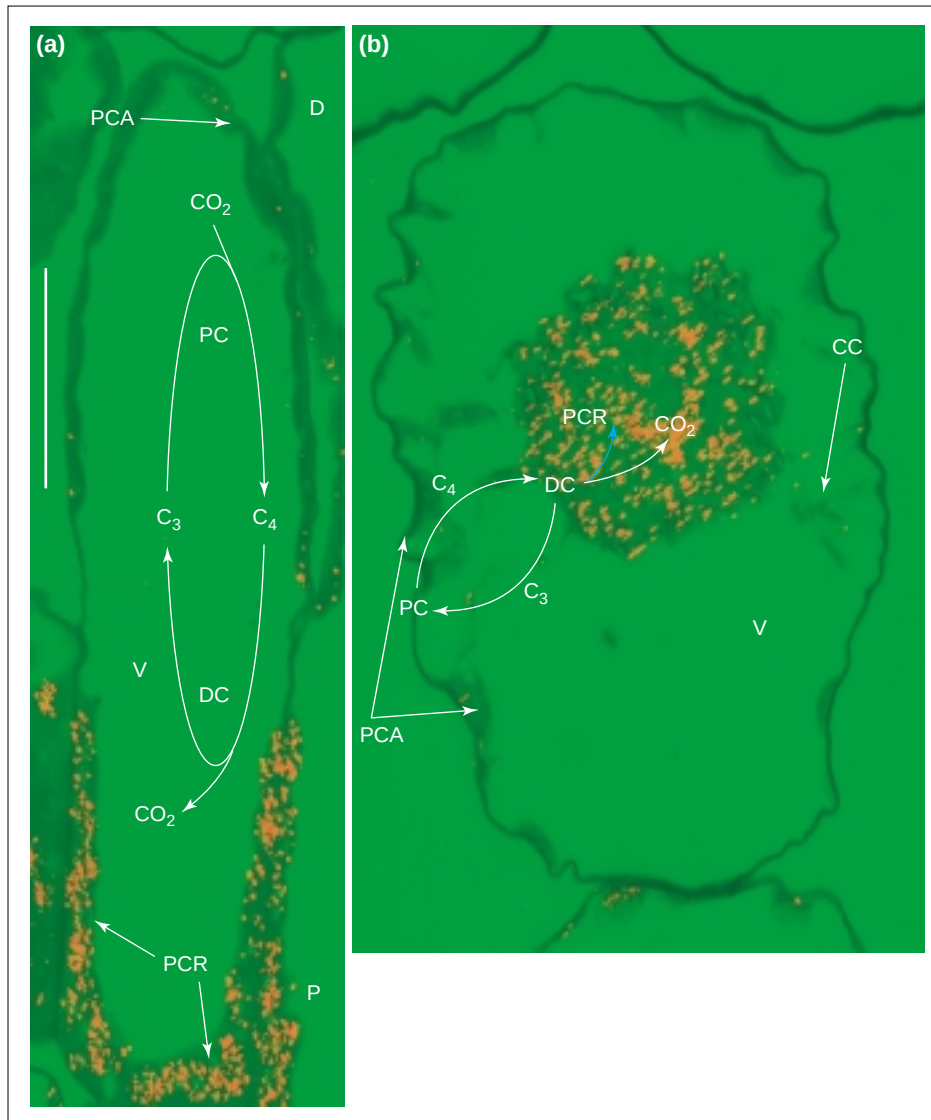


Fig. 2. (a) A photosynthetic cell from *Borszczowia aralocaspica* with immunolocalization of Rubisco at the proximal (P) end of the cell [indicated by photosynthetic carbon reduction (PCR) and the red chloroplasts; proximal is in reference to the location of the vascular bundles]. Pyruvate, P-dikinase containing chloroplasts [indicated by photosynthetic carbon assimilation (PCA)] are located at the distal (D) end of the cell. PC refers to PEP carboxylation and DC refers to decarboxylation of C₄ acids. (b) A photosynthetic mesophyll cell from *Bienertia cycloptera* showing an inner cytoplasmic compartment (PCR) connected by cytoplasmic channels (CC) to the peripheral cytoplasm. Rubisco is localized in chloroplasts in the central compartment (indicated by red coloration) surrounded by the vacuole (V). C₄ cycles are indicated for both species, where PEP carboxylation forms C₄ acids in the PCA region. These are decarboxylated in the PCR region, forming a C₃ acid and releasing CO₂. Scale bar = ~25 μ m. Photographs courtesy of Elena Voznesenskaya, Vince R. Franceschi and Gerry E. Edwards.

that every terrestrial C₄ species previously examined has some form of Kranz anatomy, the discovery of Kranz-less, or single-cell C₄ photosynthesis in terrestrial plants is a surprise.

Single-cell C₄ photosynthesis in terrestrial plants

Single-cell C₄ photosynthesis in a terrestrial setting was first detected in *B. aralocaspica*, a semi-succulent member of the Chenopodiaceae (subfamily Salsoloideae, tribe Suaedeae) from saline deserts of central Asia [4,12]. Ecologically, *B. aralocaspica* is an extreme halophyte that forms monospecific stands on the margins of ephemeral saline lakes [12]. Superficially, *B. aralocaspica* exhibits an internal anatomy resembling the salsoid type of Kranz anatomy common in the Salsoloideae. On close inspection, there are no periclinial walls separating PCA and PCR regions, yet *B. aralocaspica* exhibits a C₄-like carbon isotope signature [4,12]. CAM photosynthesis can also cause C₄ isotopic signatures and, given the succulent leaves, CAM remained a possibility until a physiological study by Elena Voznesenskaya and colleagues

unequivocally showed single-celled C₄ photosynthesis in *B. aralocaspica*. [4]

The photosynthetic cells of *B. aralocaspica* are modified, segregating PCA and PCR functions in a similar way to that found in Kranz tissue [4]. Instead of occurring in distinct cells, PCA and PCR metabolism occur at opposite ends of the same elongated cell (Fig. 2a). PEP regeneration occurs in chloroplasts located at the distal end of the cell away from the vascular bundles, whereas Rubisco and PCR functions are localized at the proximal end of the cell near the vascular bundles. In the leaf, photosynthetic cells are radially arranged around a central cylinder composed of enlarged water storage cells and numerous vascular bundles. The proximal ends of the photosynthetic cells are tightly packed with no obvious exposure to the intercellular air spaces, whereas the distal ends are directly exposed. PEP carboxylase (PEPCase) is not localized at the outer periphery of the cells but is found throughout the cytoplasm. This indicates that segregation of PCA and PCR metabolism does not involve the compartmentation of PEPCase, but instead is accomplished by the localization of PPDK (pyruvate, phosphate dikinase), Rubisco and the decarboxylating enzymes.

Carbon isotope values of *B. aralocaspica* are well within the range exhibited by C₄ plants [4,12], showing that CO₂ leakage is low. Leakage of CO₂ is minimized by the radial arrangement of the elongated photosynthetic cells, which create a long diffusive pathway for CO₂ efflux. In addition to the structural adjustment, the biochemical allocation of PCA and PCR enzymes is shifted in a manner that appears to reduce leakage. In *B. aralocaspica*, Rubisco activity relative to PPDK activity is double that observed in a related C₄ species, *Salsola laricina*. Furthermore, the ratio of PEPCase to Rubisco activity in *S. laricina* was near 18, whereas it is ~6 in *B. aralocaspica*. One might think that this ratio should be higher in *B. aralocaspica* given the need for PEPCase to recover CO₂ leaking out of the cell interior. Interestingly, *B. aralocaspica* had low activities of decarboxylating enzymes. The total activity of NAD-malic enzyme was 25% of that observed in *S. laricina* [4].

The single-celled C₄ system of *B. aralocaspica* appears to have shifted

the allocation of PCR and PCA enzymes so that the Rubisco CO₂ sink in the proximal region of the cell is enhanced relative to the C₄ cycle activity. As a result, proportionally more of the CO₂ that is pumped in can be immediately fixed before leaking out. In addition, CO₂ levels around Rubisco might be lower than in most C₄ species, therefore the concentration gradient driving CO₂ leakage would be reduced. Consistently, *B. aralocaspica* exhibited a higher CO₂ saturation point than *S. laricina*. The initial slope of the photosynthetic CO₂-response curve, which reflects the CO₂ pump strength, is also reduced in *B. aralocaspica* relative to that observed in *S. laricina* [4].

The discovery of single-celled C₄ photosynthesis in *B. aralocaspica* led to investigations of the related species *Bienertia cycloptera* (tribe Suaedeae), which also grows in extreme saline habitats in central Asia [5,6]. *Bi. cycloptera* is an oddity because it shows a C₄ isotopic signature and yet was identified as C₃ in an earlier biochemical study [5,13]. *Bi. cycloptera* is one of the most interesting C₄ species studied to date. It operates a single-celled C₄ pathway using a novel cellular arrangement that is markedly different from *B. aralocaspica* or any other C₄ species (Fig. 2b) [5,6]. Instead of the elongated Kranz-like separation of PCA and PCR functions, as occurs in *Borszczowia*, the cytoplasm of *Bi. cycloptera* is divided into a region at the cell periphery, where PCA functions are localized, and into a region in the center of the cell, where PCR functions are localized (Fig. 2b) [6]. Surrounding the central compartment is a large vacuole that is dissected by cytoplasmic strands connecting the central and peripheral cytoplasm. The vacuole appears to be the resistant barrier minimizing CO₂ efflux, and the cytoplasmic strands are the channels for metabolite flux.

Suaedeae and the evolution of C₄ photosynthesis

With the discovery of two single-cell C₄ species in the Suaedeae, this tribe has become one of the most fascinating groups for C₄ plant biologists. The Suaedeae comprises four genera: *Suaeda* (100 species, 60% C₄), *Alexandra* (one C₃ species), *Bienertia* (one C₄ species), and *Borszczowia* (one C₄ species) [12–14]. Within the Suaedeae, five distinct C₄ origins are suspected – three in *Suaeda*, and one

each in *Bienertia* and *Borszczowia* [5]. Why are the Suaedeae so prolific at evolving C₄ photosynthesis? The answer is probably associated with the extreme saline soils where Suaedeae species grow. High salinity restricts interspecific competition and favours characteristics enhancing water-use efficiency. Thus, novel traits that improve water-use efficiency, such as single-cell C₄ photosynthesis, might have had a competitor-free space in which to evolve.

In recent years, there has been much interest in turning C₃ crops, such as rice, into C₄ plants [15,16]. The major barriers to this goal are probably not biochemical but structural. C₃ species already have the necessary enzymes for C₄ photosynthesis, and some, such as tobacco and celery, can even operate a version of the C₄ cycle between roots and vascular parenchyma cells of photosynthetic stems [17]. The more significant hurdle is thought to be engineering Kranz leaf anatomy to allow for CO₂ concentration. With the discovery of single-celled C₄ photosynthesis, the task of engineering C₄ plants from C₃ species might be easier than previously thought [16]. Because these species show how C₄ photosynthesis can work in single cells, the obscure desert plants *Bienertia* and *Borszczowia* might be key to efforts to introduce the C₄ pathway into C₃ crops.

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