Does starch influence time keeping in CAM plants?

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Introduction

In crassulacean acid metabolism (CAM) CO_2 uptake primarily takes place overnight, reducing water loss [1]. In water deficient environments daytime photosynthesis would result in detrimental water loss. Therefore, desert-living plants such as cacti and aloe have evolved CAM [2]. CAM plants use the circadian clock to tell the time; thus, the clock is essential to ensure photosynthesis occurs at night.

The ultimate objective is to bioengineer CAM into traditional crops in order to reduce water requirements, enable the expansion of agriculture in arid environments, and sustain plant productivity to match worldwide food and biofuel demands [3]. However, this will require greater understanding of CAM photosynthesis, including factors that may influence temporal regulation by the circadian clock, such as leaf starch.

Aims

This project aimed to investigate whether a lack of starch:

- Reduces the efficiency of nocturnal photosynthesis
- Reduces the ability to photosynthesise nocturnally following lengthened and shortened photoperiods, in CAM plants

Methods

The research used wild type (WT) and phosphoglucomutase (PGM) mutants, which have a lack of leaf starch, of the CAM plant *Kalanchoë fedtschenkoi*. Several factors involved in CAM photosynthesis were investigated, with measurements of:

- Net nocturnal CO_2 assimilation, following either shortened or lengthened photoperiods – measured by infrared gas analysis.
- Malic acid content as titratable acidity (a biochemical marker for CAM) – measured from NaOH titrations.
- Starch and soluble sugar content – measured by colorimetric phenolic-sulphuric acid tests.
- Phospho*enol*pyruvate carboxylase (PEPC) protein content – determined from western blotting.
- Plant growth

Results – CO_2 assimilation

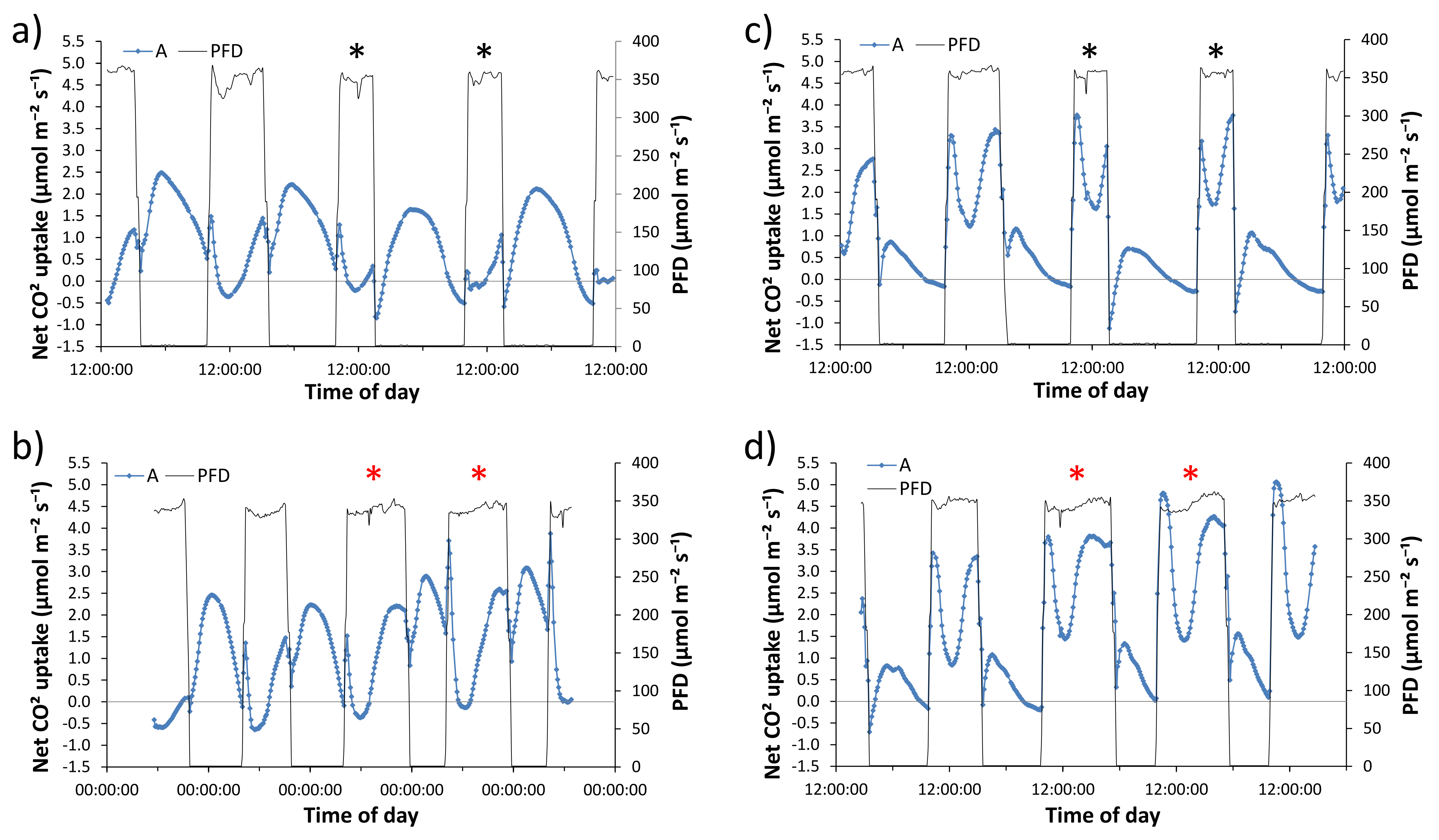

Figure 1. Net CO_2 uptake in WT (a, b) and PGM mutant (c, d). Photoperiod durations of 8 h (*) and 16 h (*) are marked. A: CO_2 assimilation; PFD: photon flux density.

The PGM mutant assimilated most of its CO_2 during the day, in contrast to the WT plants. Statistically, the WT adjusted well to both 16h and 8h photoperiods, as the net CO_2 uptake did not differ significantly following the photoperiod change; whereas the PGM only adjusted its CO_2 uptake appropriately following the 8h photoperiod, and assimilated significantly more CO_2 when subjected to a 16h photoperiod.

Results – Malic acid

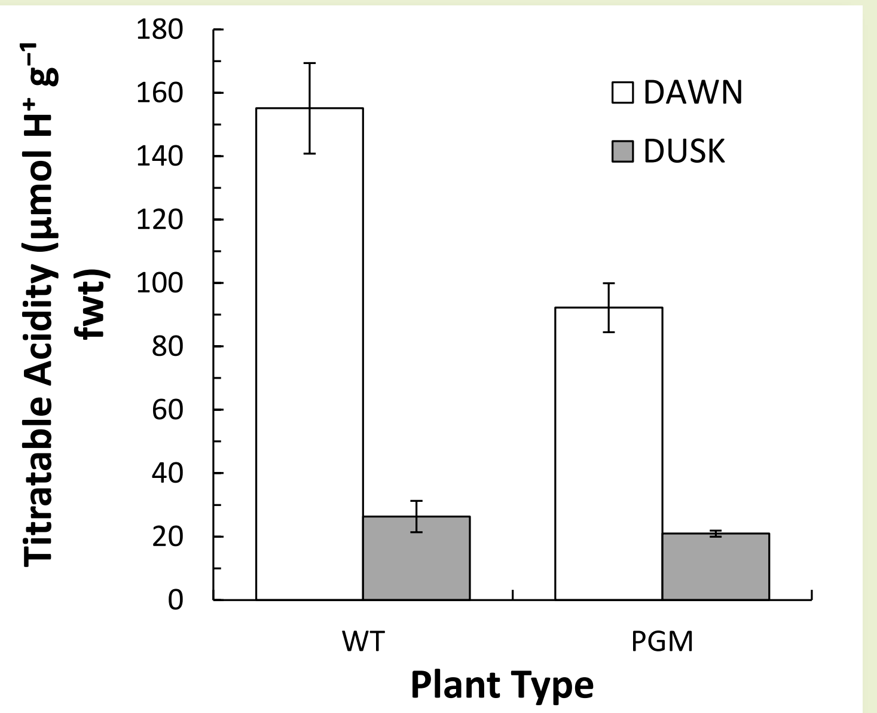

Figure 2. Titratable acidity in WT and PGM mutant plants, at dawn and dusk.

Malic acid increased overnight in both plant types; this indicates that CAM is occurring. However, the PGM mutant produced approximately 45% less acidity than the WT.

Results – Carbohydrate

Leaf starch content increased during the day in the WT only, as expected.

Inversely, soluble sugar content only increased in the PGM mutant.

Results – Growth and PEPC Protein

Figure 3. WT (left) and PGM mutant (right) *Kalanchoë fedtschenkoi* plants.

Growth was significantly reduced in the PGM mutants, in regards to: height; total leaf area; and total leaf fresh and dry weights.

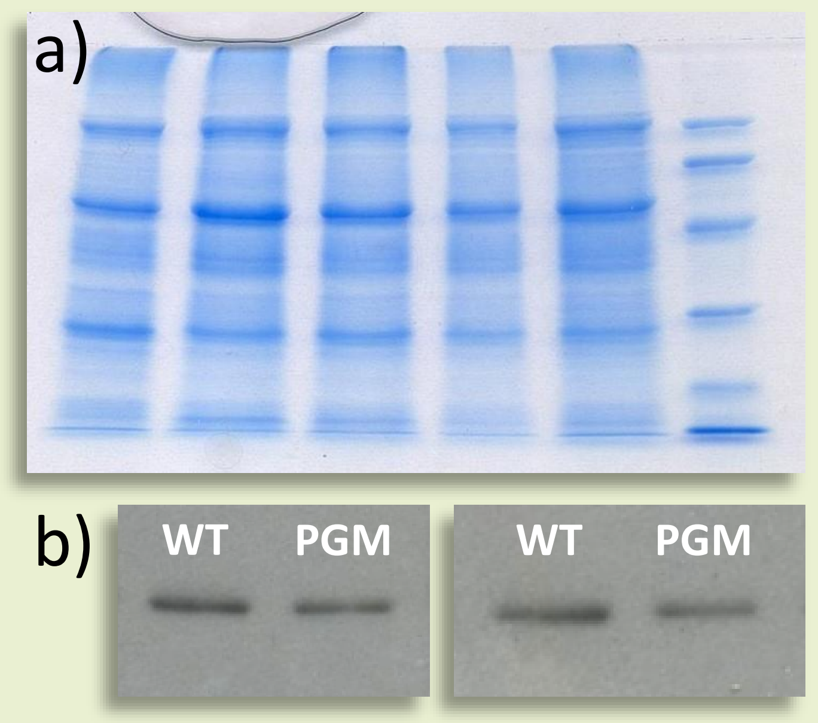

Figure 4. (a) An example gel containing proteins separated by size. (b) Two examples of WT and PGM mutant plants and their respective PEPC bands.

PEPC content was markedly higher in the WT compared to the PGM mutant.

Discussion

- The PGM mutant uses soluble sugars as its carbohydrate store, instead of starch, at the detriment of plant growth
- Malic acid production and PEPC content was much lower in the PGM mutant, therefore nocturnal uptake of CO_2 was limited; the PGM mutant compensated by assimilating more CO_2 during the day
- As the PGM mutant predominantly assimilated CO_2 during the day water loss will be much greater than in the WT
- A lack of starch negatively impacts the regulation of CO_2 uptake by the circadian clock, following an extended photoperiod

This suggests that a store of starch in the leaf will be required to facilitate the bioengineering of CAM into food crops and for water-efficient nocturnal photosynthesis.

Acknowledgments

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References

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