Modulation of Carbon assimilation and induction of Crassulacean acid metabolism (CAM)

Erin Casey* and Dr Tahar Taybi, School of Biology, Newcastle University.

Aim

To gain understanding into how Crassulacean Acid Metabolism is induced in the plant *Kalanchoe blossfeldiana, var.* kerenzi a facultative CAM plant which switches from C3-photosynthesis to CAM when under water stress.

Introduction

Today many crops are failing due to lack of water; this is increasing food prices in the west, whilst many starve in less economically developed countries. Through the development of genetically engineered crop plants, increased food security would be available.

Crassulacean acid metabolism (CAM); is a drought-adapted type of photosynthesis which reduces photorespiration and water loss through fixing CO_2 at night. This offers a great opportunity for crop development if genetically engineered plants could be grown in a more arid environments. First however greater understanding as to what underpins the mechanisms of CAM is needed.

In contrast to C3- and C4-plants, CAM plants open their stomata at night in order to absorb and assimilate atmospheric CO_2. This is done using phosphoenolpyruvate carboxylase (PEPC) to produce organic acids (malate). Stomata then close most of the day to prevent water loss and the CO_2 stored during the night is released by decarboxylation of malate. This is then assimilated by Ribulose bisphosphate carboxylase oxygenase (RUBISCO) via the Calvin cycle to produce carbohydrates.

The CAM cycle has great plasticity and facultative CAM plants have the ability to switch between C3 and CAM when water stress; however the way this process occurs is largely unknown.

Here we investigated the process underpinning the switch from C3 to CAM in the facultative CAM plant *K. blossfeldiana*.

Objectives

We investigated the switch from C3 to CAM using the following objectives:-

- **CO2 - exchange measurement** to confirm the switch of carbon assimilation to CAM under water stress.
- ***PEPC* transcript monitoring using semi-quantitative RT-PCR** in *K. blossfeldiana* under C3 and CAM to diagnose CAM-induction at the gene level.
- **Isolation of Ca^{2+}-dependant protein kinase genes** using degenerate RT-PCR from *K. blossfeldiana* under C3 and CAM as potential mediators of CAM-induction.

References

Gehrig H, Taybi T, Kluge M, Brulfert J, 1995. Identification of multiple PEPC isogenes in leaves of the facultative crassulacean acid metabolism (CAM) plant *Kalanchoe blossfeldiana* Poelln. cv. Tom Thumb. *Febs Letters* **377**, 399-402.

Results

Gas Exchange

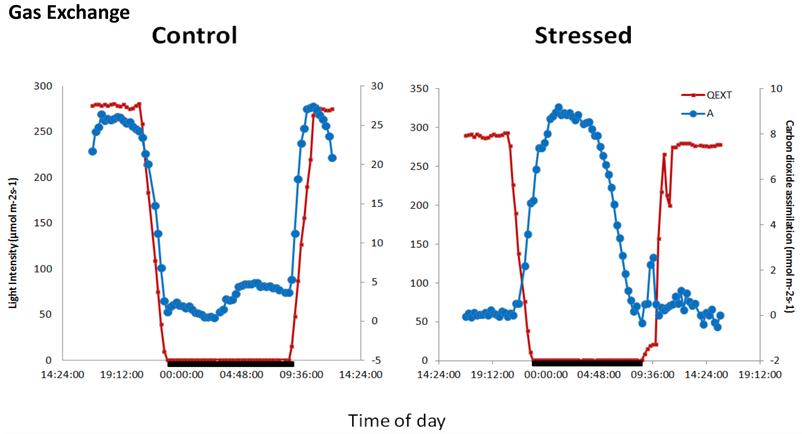

Figure 1. Gas exchange results for the control and stressed plants. The black bar indicates night. QEXT denotes light intensity and A denotes CO_2 assimilated.

In the control plant it can be observed that as light reduces the rate of CO_2 uptake decreases, indicating that the stomata have shut (Fig. 1).

Whereas in the water– stressed plant the reverse can be observed, where as light levels decrease CO_2 uptake increases.

However it can be noted that in the control plant a small amount of CO_2 fixation occurred at night which might be the consequence of respiratory CO2-recycling by PEPcarboxylase.

PEPC concentration

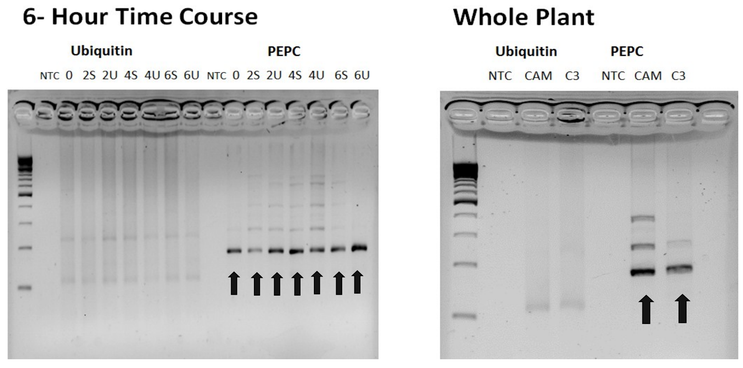

Figure 2. PEPC transcript levels found in CAM and C3 phenotypes of the whole plant and over a 6-hour detached leaf time course, where control and stressed leaves were tested.

Transcript levels for *PEPC1* gene were monitored in detached leaves and whole plants subjected to water stress. As shown in figure 2, water-stress caused no significant increase in *PEPC1* transcripts in both detached leaves and whole plants. Ubiquitin gene (Ubq) was used as control.

Protein kinases isolated from *K. blossfeldiana*

Clone 1: Serine/Threonine protein kinase:-

GAGATCTGAAGCTGGAAAACGGGTCACGGAAAGAATCATGAGAATTTAGGCTTGGGCATCGGGGTGGTGGC
GGGGGAGTGCACGGGGCCGAGCTTCTGGATCCAGTGAAGAAGTTTCTTTTTGTGATCTTGACAGATGGGGTT
GTGTGCGAGGCTCAAAGTCCCGTCACACGCGAGAGAGGTTGCGAATTGTTCCAGGACCTTCACGAGTTGCCA
GAAGTCCGGCCGCTTGTCCGGATGTGAGGACCAGCATTGCTCTATCAGGGCTTTCATGGCCGGAGGGCAGTC
CTCGGGGATGGCAGGTCGCAGCTTCTTATTGACTACGGCGAACGCAGCTTGTATTGGATTCATGTCCTCGTAA
GGGATGGACCCGGATATCATCTCCCACAAGATGAGCCCGAAGCTGTACACGTCGACCTTCTTCCCGTACGACT
TGTGTTTGATCATTTCGGGAGCCATCCAACGGTAGGTGCCCGGATCATCAGCCAAGGCATCACAATAGGCCTC
CTCGCAAGCTATCCCGAAATCCGCAATTTTCATGCGGAAGTCTTGATCGATAAGGATGTTTTCCAGCTTTAGAT
CGC

Clone 2: Receptor like Serine/Threonine protein kinase:-

GGGATCTAAAGCTCGAAAACCAGTGCCAAGAATTTGTTACCCTTGTCGTCAATACTTGAACATACGGTTAAAA
TCTTAAGAATATTTCTATGGCGGACAGCTCCAAGAGATTGGCATTCTGCATTGAAGGTTTTTAAAGCGCCATGT
TTCTGAAGGTTCAATACCTTCACTGCAACCGGTTTCTGTTCTTCAAACAAAAATCCCTTGTAAACAGATCCCATA
CTTCCCATGCCAATCAAGTTGGATGAATCAAATCCGGATGTTGCATCATGAAGGTCTTTGTATGAGACTCTCAA
GAGCCCGTTTCCCTCGAAAGAAGCCAAAGATGACTGCCTCTTCACCTTCCTTTTCCAGCTTCAGATCCC

Figure 3. The sequences of protein kinases isolated from *K. blossfeldiana.*

Two protein kinases were isolated from CAM induced detached leaves. The first was found to be a Serine/Threonine protein kinase and the second was a receptor like Serine/ Threonine protein kinase.

Conclusions and Discussion

- CAM was induced in *K. blossfeldiana* when subjected to water stress (Figure 1).
- *PEPC* transcript levels remained unchanged in leaves under water-stress (Figure 2). This could be due to a constitutive up regulation of this key CAM gene in both the C3 and CAM state; suggesting that this variety of *K. blossfeldiana* might be an intermediate inclined towards constitutive CAM. Or simply that a housekeeping *PEPC* isogene was targeted by the RT-PCR assay, as four different *PEPC* isogenes have been characterised in this species (Gehrig et al., 1995).
- Two protein kinases were isolated from the CAM induced detached leaves (Figure 3.) one is a Serine/Threonine protein kinase and the second is a receptor like Serine/ Threonine protein kinase. The isolated proteins could regulate the shift from C3 to CAM under water stress.

This investigation has revealed that varieties of *K. blossfeldiana* might be intermediates between facultative CAM and constitutive CAM and thus they might be valuable tools for understanding CAM evolution. Further investigation should determine if the isolated protein kinase genes play a role in CAM expression.

Acknowledgements

I would like to thank Dr Tahar Taybi, Dalal Al– Baijan and all EMPP lab staff who facilitated this project. I would like to thank the Newcastle University for granting me a vacation Scholarship.

*Biology undergraduate, e.casey@ncl.ac.uk