

Clarifying assimilate transport & storage in monocot stems

Thesis submitted for the doctorate of philosophy

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Declaration:

“I hereby certify that this doctoral thesis is submitted in the form of a series of published papers of which I am joint author. I have included as part of the thesis a written statement from each co-author; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.”

William Moreau Palmer

Date:

Acknowledgements:

The years gone by have been full of amazing highs and lows. Science is inherently about failure but more importantly about learning and adapting ones thought and process to overcome these barriers. Many people are involved in this process starting with the contemporaries that I have worked with during the daily grind literally! Those of the Plant Science Group whom there are too many to name but I have shared many adventures with. An early mentor of mine Mickey who has left as now but so inspirational and formative to my foray into microscopy, miss you mate. The usual suspects including Simon, Ricky, Hannah, Chris, Kim, Fred who have come and gone. My dearest friend from my first lab in first year Lizzy, you're the best.

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To the Leah, you were my rock and all the good times we have had throughout our University life. To my lifelong friends who I have escaped to when needed and you have pulled me back up and sent me back in.

And finally my family, Dad what a time, I know you thought I would never get the bloody thing done but here it is .. finally. Mum, without you this would not ever have been possible. From beginning to end you have been there. Love you both.

Overview:

This thesis is quite diverse in nature. The first section is about three dimensional imaging, the second about high throughput phenotyping and the third genetic sequencing. It is all driven towards trying to understand how sugars are stored in stems.

Each section contained within has an explanation of how and why this diversity was conducted. It has coalesced into novel techniques, inventions and genetic targets that when combined will undoubtedly help drive understanding of the post phloem pathway.

I hope you enjoy reading it as much as I have enjoyed doing it.

List of publications included as part of thesis:

Section 1 – 3D Imaging

1. Palmer WM, Martin AP, Flynn JR, Reed SL, White RG, Furbank RT, and Grof CPL. **PEA-CLARITY: 3D molecular imaging of whole plant organs.** *Nature Scientific Reports*, 5, Article number: 13492 (2015)

- **Post Publication Peer Review F1000.**

Geldner N and Andersen T: F1000 Prime Recommendation of [Palmer WM *et al.*, Sci Rep 2015, 5:13492]. In F1000Prime, 17 Sep 2015; DOI: 10.3410.

- **Nature Plants Editorial – Recipes for Success.**

Nature Plants 1, Article number:15157 (2015) doi:10.1038/nplants.2015.157

- **2016 Australian Eureka Prize Finalist – Innovative use of technology**

William Palmer, Dr Jamie Flynn and Antony Martin have developed advances in plant-enzyme-assisted (PEA)-CLARITY tissue clearing technique, built a custom light sheet microscope and founded a new three-dimensional tissue clearing and light sheet microscopy facility. www.australianmuseum.net.au/2016-eureka-prizes-finalists

2. Palmer WM, Martin AP, Flynn JR, Reed SL, White RG, Furbank RT, and Grof CPL. **PEA-CLARITY: three dimensional (3D) molecular imaging of whole plant organs.** *Bio-protocol*, 6(21): e2000. DOI: <https://doi.org/10.21769/BioProtoc.2000>

Section 2 – High Throughput Phenotyping

3. Martin AP, Palmer WM, Byrt CS, Furbank RT, Grof CP. **A holistic high-throughput screening framework for biofuel feedstock assessment that characterises variations in soluble sugars and cell wall composition in *Sorghum bicolor*.** *Biotechnology for Biofuels*. 2013 **6**:186. doi:10.1186
4. William M Palmer, Principle Inventor. *Patent Cooperation Treaty (PCT) Patent: Multi Sample Biomass Grinder. PCT/AU2016/050810. International filing date: 29th August 2016.*

Section 3 – Post Phloem Pathway Gene Discovery

5. William M Palmer, Antony P Martin, Joshua Atkins, Simon Wheeler, Lloyd Handyside, Sam Bliss, Chris PL Grof, Caitlin Byrt, Christopher Lambrides and Robert T Furbank. **Elucidation of QTLs and candidate genes for stem carbohydrate transport and storage in sorghum using a novel phenotyping method and a genotype by sequence mapping approach.** *Sent to co-authors for review, April 2017.*
6. Martin AP, Palmer WM, Brown C, Abel C, Lunn JE, Furbank RT and Grof CPL. **A developing *Setaria viridis* internode: A new experimental system in a C₄ model species.** *Biotechnology for Biofuels* 2016 **9**:45 doi: 10.1186

Appendix

William M Palmer, Lei Ru, Ye Jin, John W Patrick, Yong-Ling Ruan. **Tomato Ovary-to-Fruit Transition is Characterized by a Spatial Shift of mRNAs for Cell Wall Invertase and its Inhibitor with the Encoded Proteins Localized to Sieve Elements.** *Molecular Plant*, 8(2):315-328. Feb 2015.

- **Molecular Plant – Front Cover**, February 2015.

William M Palmer, John W Patrick and Yong-Ling Ruan. **Resin-embedded Thin-section Immunohistochemistry coupled with Triple Cellular Counterstaining.** *Bio-protocols*, accepted for publication. Online print date 5th April 2017. Vol 7, Iss 7, April 05, 2017 <https://doi.org/10.21769/BioProtoc.2052>

Additional publications:

Palmer WM, Flynn JR, Martin AP, Reed SL, Grof CPL, White RG, Furbank RT. **Three dimensional Imaging techniques to define C₃ and C₄ photosynthesis.** *Book Chapter, Photosynthesis methods and protocols*. Springer 2017, Accepted

Li Z, Palmer WM, Martin AP, Wang R, Rainsford F, Jin Y, Patrick JW, Yang Y, Ruan YL. **High invertase activity in tomato reproductive organs correlates with enhanced sucrose import into, and heat tolerance of, young fruit.** *Journal of Experimental Botany*. Feb 2012.

Martin AP, Brown CW, Nguyen DQ, Palmer WP, Byrt CS, Lambrides CJ and Grof CPL. **Cell wall development in an elongating internode of Setaria.** *Book Chapter, Genetics and Genomics of Setaria*. 1(19) 2017.

Conference Talks:

ComBio 2014; COL-01-04. **PEA-CLARITY: 3D Immunohistochemistry of Whole Mount Plant Tissues.** Palmer WM, Martin AP, Furbank RT and Grof CPL. Canberra, ACT, Australia.

Conference Posters:

American Society of Plant Biologists (ASPB July-2015). **PEA-CLARITY: 3D Molecular Imaging of Whole Plant Organs.** Palmer WM, Martin AP, Furbank RT and Grof CPL. Minneapolis, Minnesota, United States of America.

ComBio 2014; POS-MON-031. **Molecular Characterisation of a Developing *Setaria viridis* Internode.** Martin AP, Palmer WM, Lunn JE, Mouille G, Stitt M, Furbank RT and Grof CPL. Canberra, ACT, Australia.

ComBio 2014; POS-MON-187. **Whole Tissue Clearing Using the CLARITY Technique.** Flynn JR, Martin AP, Palmer WM, Rank M, Callister R and Smith K. Canberra, ACT, Australia.

European Plant Science Organisation (EPSO Sep-2013). **A holistic high-throughput screening framework for biofuel feedstock assessment that characterises variations in soluble sugars and cell wall composition in *Sorghum bicolor*.** Martin AP, Palmer WM, Furbank RT and Grof CPL. Porto Heli, Greece.

ComBio 2012; POS-WED-049. **Regulation of fruit set by cell wall invertase: Phloem-specific localization and activity burst during ovary-to-fruit transition.** Palmer WM, Jin Y, Ru L, Wang L, Patrick JW and Ruan Y-L. Canberra, ACT, Australia.

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1.2 Methodological Article

Palmer WM, Martin AP, Flynn JR, Reed SL, White RG, Furbank RT, and Grof CPL. PEA-CLARITY: three dimensional (3D) molecular imaging of whole plant organs. Bio-protocol, 6(21): e2000. DOI:10.21769

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2.1 Research Article

Martin AP, Palmer WM, Byrt CS, Furbank RT, Grof CP. A holistic high-throughput screening framework for biofuel feedstock assessment that characterises variations in soluble sugars and cell wall composition in *Sorghum bicolor*. *Biotechnology for Biofuels*. 2013 6:186. doi:10.1186

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2.2 Patent

William M Palmer, Principle Inventor. Patent Cooperation Treaty (PCT) Patent: Multi Sample Biomass Grinder. PCT/AU2016/050810. International filling date: 29th August 2016

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3.2 Research Article

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Research Article

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Immunolocalisation protocol

William M Palmer, John W Patrick and Yong-Ling Ruan. Resin-embedded Thin-section Immunohistochemistry coupled with Triple Cellular Counterstaining. *Bio-protocols*, accepted for publication. Online print date 5th April 2017. Vol 7, Iss 7, April 05, 2017 <https://doi.org/10.21769/BioProtoc.2052>