



Australian Government
Cotton Research and
Development Corporation

TRAVEL, CONFERENCE or SCIENTIFIC EXCHANGE REPORT 2015

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: CRDC1522

Project Title: Travel: Collaborative Research with USDA Lubbock
and attendance at the Plant Biology 2015 meeting.

Project Commencement Date: 25-7-15 Project Completion Date: 8-8-15

CRDC Research Program: 2 Industry

Part 2 - Contact Details

Administrator: Sara Shortt -RSB Grants Officer
Organisation: Australian National University
Postal Address: RN Robertson Building 46, The Australian National University
Acton ACT 2601

Ph: 02 6125 8384 Fax: E-mail: sara.shortt@anu.edu.au

Principal Researcher: Dr Robert Sharwood - ARC DECRA Fellow.
Organisation: Australian National University
Postal Address: Building 134 Linnaeus Way, Acton ACT 2601

Ph: 040207224 Fax: E-mail: robert.sharwood@anu.edu.au

Supervisor: Prof. David Tissue
Organisation: Western Sydney University, Hawkesbury Institute for the
Environment

Postal Address: Locked Bag 1797 Penrith NSW 2751.
Ph: 02 4570 1853 Fax: E-mail: d.tissue@westemsydney.edu.au

Signature of Research Provider Representative: _____

(I/rt) (LotS)

Part 3-Travel, Conference or Scientific Exchange Report

(Maximum twopages)

1. A brief description of the purpose of the travel.

Travel to Lubbock Texas USA to meet with Dr Paxton Payton and Dr James Mahan at the USDA-ARS to begin collaborative research to determine the interactive effects of water deficit and elevated temperature with elevated [CO₂] on cotton growth and physiology, and attend the Plant Biology 2015 meeting taking place at Minneapolis, Minnesota United States to communicate my recent research findings arising from the Science and Innovation award project and subsequent follow up experiments.

2. What were the:

- a) major findings and outcomes
- b) other highlights

Plant Biology 2015 – At this conference I presented my latest Cotton Research as a poster presentation (see attached presentation). This conference crossed many disciplines of plant research including plant developmental regulation, engineering photosynthesis, plant biochemistry and ecophysiology. Attendees were particularly interested in my research regarding the first interrogation of Cotton Rubisco kinetics (the key enzyme of CO₂ fixation) and the nitrogen partitioning into Rubisco synthesis. Researchers were also interested in carbon isotope discrimination and how plant breeding was able to increase entry of CO₂ into the chloroplasts. There were other posters on cotton research which enabled me to see what was happening within the field.

Visit to USDA Lubbock – I met with Paxton Payton and James Mahan who are senior cotton researchers in the USA with strong links with the Australian Cotton Industry. We had a series of meetings concerning future cotton research with Prof. David Tissue from Western Sydney University. We designed three experiments: one will be done in Lubbock and the other two in the field at ACRI Narrabri and Western Sydney University. An exciting highlight of this meeting was that I forged a strong collaboration with Paxton Payton and have now begun some research together. The aim is to overexpress the enzyme seduheptulose, 1,7 biphosphatase in their USA cotton transformable lines. This enzyme has previously been shown in other plants to influence carbon cycling in the plant and over expression resulted in improved plant biomass. Finally, this meeting allowed me to get a more complete insight into cotton research at the USDA, which will ensure crucial new information will be available to the cotton industry in Australia. On the last day, I gave a presentation to the Mahan and Paxton Research groups that outlined my future research program involving cotton and possible avenues to improve cotton photosynthesis by molecular engineering.

3. Detail the persons and institutions visited, giving full title, position details, location, duration of visit and purpose of visit to these people/places.

Dr James Mahan -United States Department of Agriculture – Agricultural Research Services, Lubbock, 7 Days - Cotton Research Collaboration

Dr Paxton Payton – United States Department of Agriculture -Agricultural Research Services, Lubbock, 7 Days - Cotton Research Collaboration

Dr John Burke – United States Department of Agriculture -Agricultural Research Services, Lubbock, 1 Day - Sorghum and Cotton Research collaboration.

Prof. David Stem -Met at Plant Biology – 1 Day - CEO Boyce Thompson Institute, Cornell University, Ithaca NY. Strategies for Improving Photosynthesis.

Prof. Tom Brutnell - Met at Plant Biology – 1 Day - Biofuels Director, Danforth Plant Science Center St Louis USA. Strategies for Improving Photosynthesis.

Prof. Martin Parry Met at Plant Biology – Director, Rothamsted Research United Kingdom. Engineering improvements in photosynthesis.

Dr Rao Kottapalli -Texas Tech University -Research Associate Professor, Center for Biotechnology & Genomics – Proteomics and RNA sequencing of cotton.

4. a) Are there any potential areas worth following up as a result of the travel?

There are a number of areas that will be followed up through this collaborative visit:

- i) With Paxton (USDA) and Rao (Texas Tech) experiments involving proteomics and RNA sequencing will be conducted on cotton genotypes. This will be invaluable to the Australian Cotton Industry to give valuable insight to cotton gene and protein regulation.
- ii) Following up transgenic testing of DNA constructs to overexpress important photosynthetic genes within cotton in collaboration with Paxton Payton.
- iii) Joint experiments between CSIRO Narrabri, USDA Lubbock, Western Sydney University and Australian National University will be followed up in the near future. I will participate in these experiments by analysing the biochemistry of photosynthetic proteins within cotton chloroplasts to interrogate the ways to improve cotton yields under future uncertain climates.

b) Any relevance or possible impact on the Australian Cotton Industry?

These areas outlined above will significantly impact the Australian Cotton Industry by improving our knowledge of the response of cotton photosynthesis under future climates. Through genetic manipulation of cotton, the aim is to improve the resilience of cotton photosynthesis under extreme temperatures to mitigate possible yield penalties.

5. How do you intend to share the knowledge you have gained with other people in the cotton industry?

Recently, I shared this knowledge during my presentation at the 2nd Australian Cotton Research Conference in Toowoomba. I was able to communicate more in depth through interactions with researchers and growers after my talk during the meeting breaks. I plan to be in regular contact with Dr. Michael Bange (CSIRO Narrabri) and Prof. David Tissue (Western Sydney University) to update them on the progress of my research.

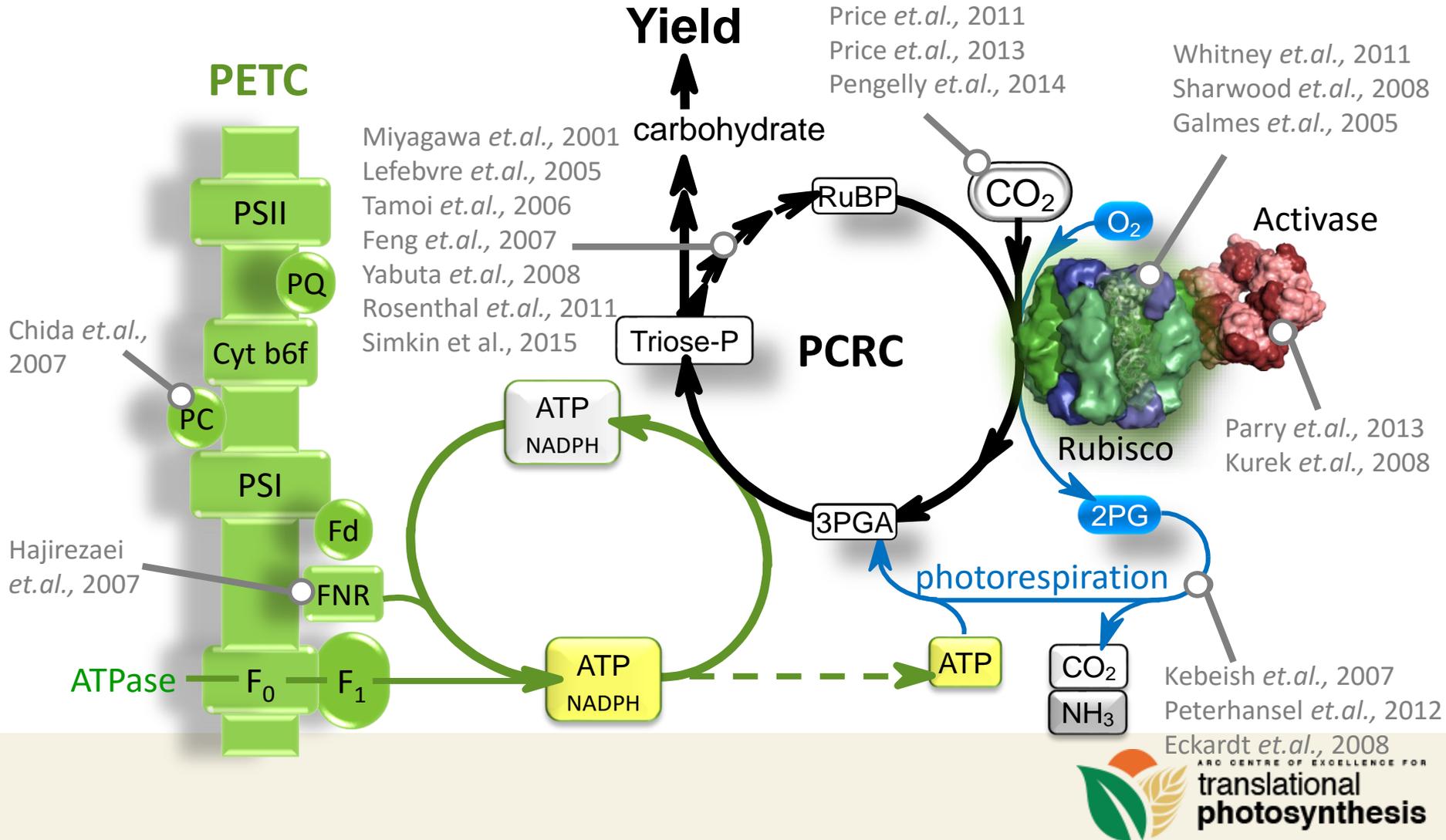
6 Please list expenditure incurred. (*Double click inside the table to enter the data*)

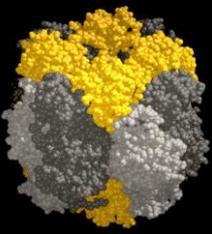
Exploring the potential translational photosynthetic applications in cotton

Dr Robert Sharwood
ARC DECRA Fellow – ANU



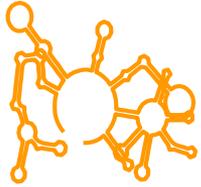
Prospects for increasing photosynthesis





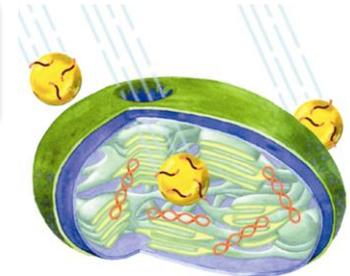
Research Program at ANU

Two Main Research themes:



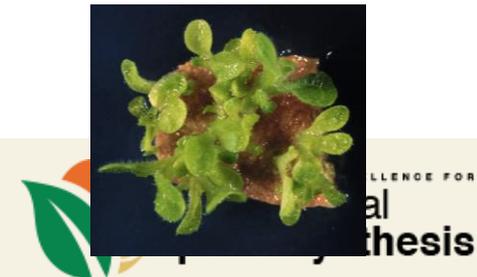
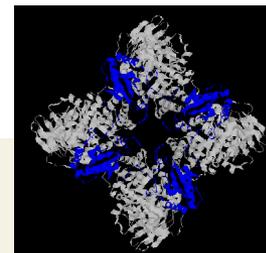
Post-transcriptional RNA metabolism

- Influence of the nucleus on chloroplast gene regulation.
- Study of ribonucleases and RNA binding proteins targeted to the chloroplast.
- RNAseq to characterise cell type gene expression in C₄ plants.



Photosynthetic biochemistry

- Rubisco bioengineering by plastid transformation.
- Rubisco biochemistry to characterise the performance of different forms.
- Developing methods for studying Rubisco within different organisms.

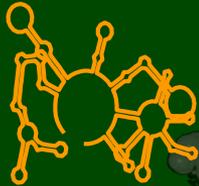


Biochemistry and Molecular Biology of Photosynthesis

'Understanding the impact of global climate change on photosynthesis'

Chloroplast Gene Regulation

- RNAseq to understand nuclear-chloroplast gene regulatory elements
- Funding: ARC DECRA Fellowship



Eucalyptus photosynthesis

- Thermal acclimation of photosynthetic enzymes
- Leaf level nitrogen optimization
- Rubisco kinetic screen
- Gene sequencing



Cotton Photosynthesis

- Water use efficiency
- Thermotolerance
- Biochemistry of photosynthesis
- DAFF Science and Innovation - Mick Bange and David Tissue
- FUNDING - CRDC



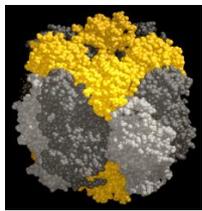
Rubisco Biochemistry

- Screening for catalytic diversity
- Finding critical catalytic residues
- Determining the effect of temperature on kinetic properties

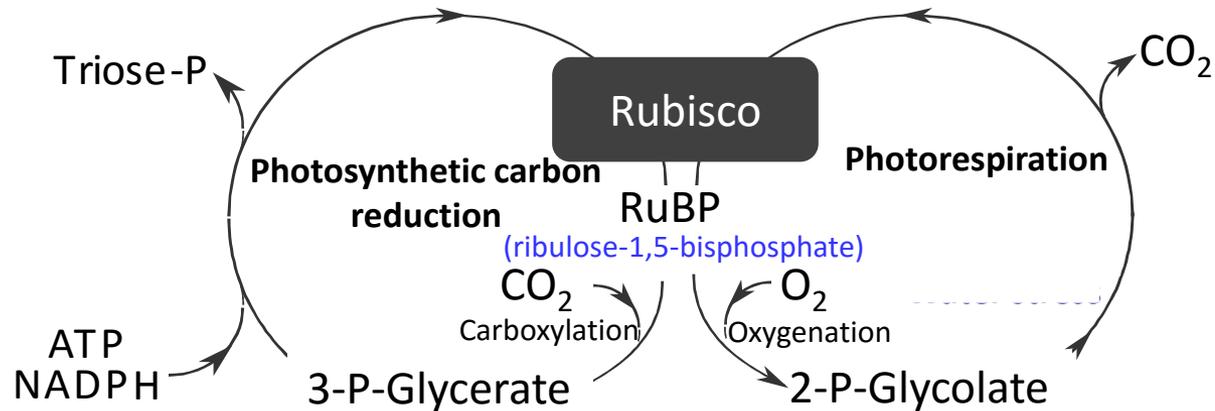


Physiology and Transformation of C₄ grasses

- Determining the efficiency of the CO₂ concentrating mechanism
- Investigating N and C efficiency
- Molecular transformation of Setaria - a model C₄ grass



Bifunctional Rubisco catalysis and photorespiration



1) Complexity of catalysis:

- Requirement for activation
- Catalytic chemistry beginning from the enediol
- Maintenance of activity by Rubisco Activase

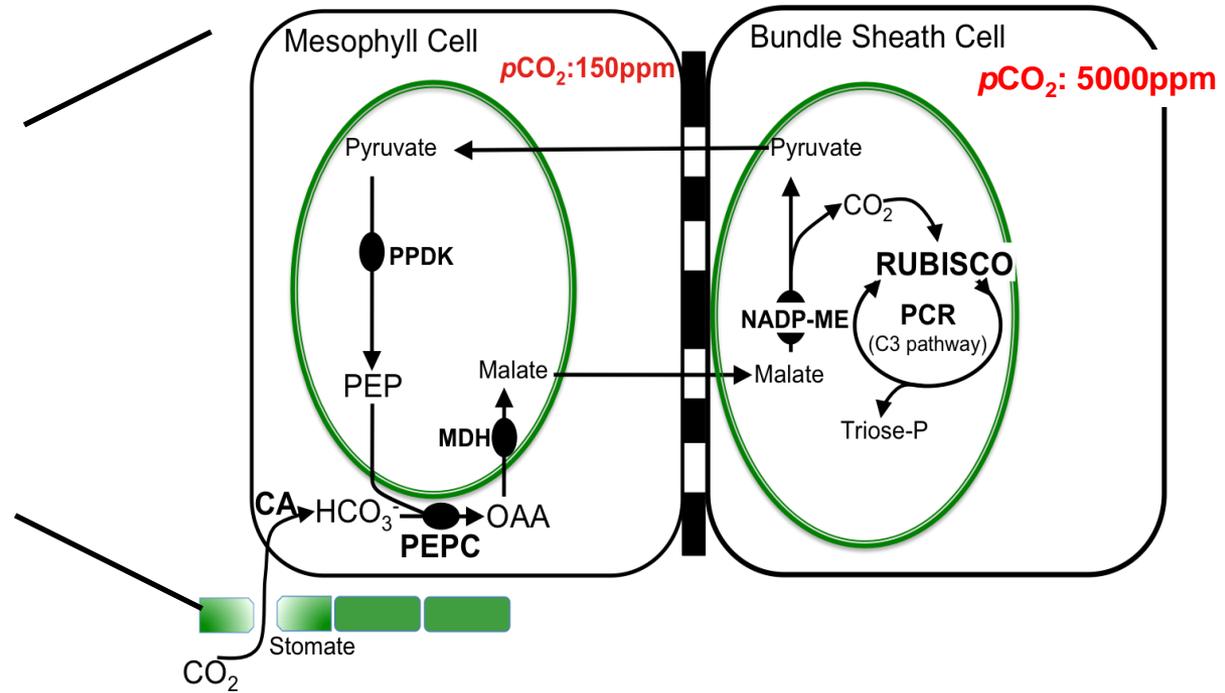
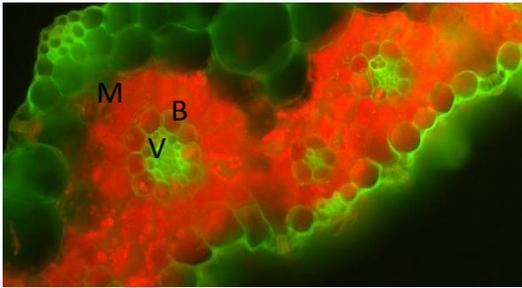
2) Catalysis: there is room for improvement

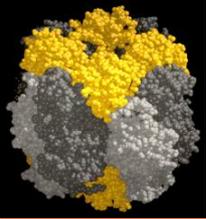
- specificity ($S_{c/o} = v_c / v_o$) for CO₂ as opposed to O₂ is low
- slow catalytic turnover ($k_{cat}^c \sim 1 - 3 \text{ (s}^{-1}\text{)}$)
- several abortive side reactions

C₄ photosynthesis circumvents Rubisco inefficiency

NADP-ME subtype:

Kranz Anatomy





Rubisco catalytic parameters

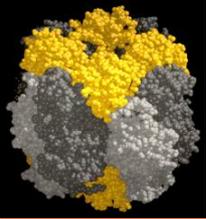
k_{cat}^c (s^{-1}) : Rubisco catalytic turnover speed for CO_2 fixation.

$K_m(CO_2)$ (μM) : Rubisco affinity for CO_2

Lack of comprehensive datasets for Rubisco catalysis

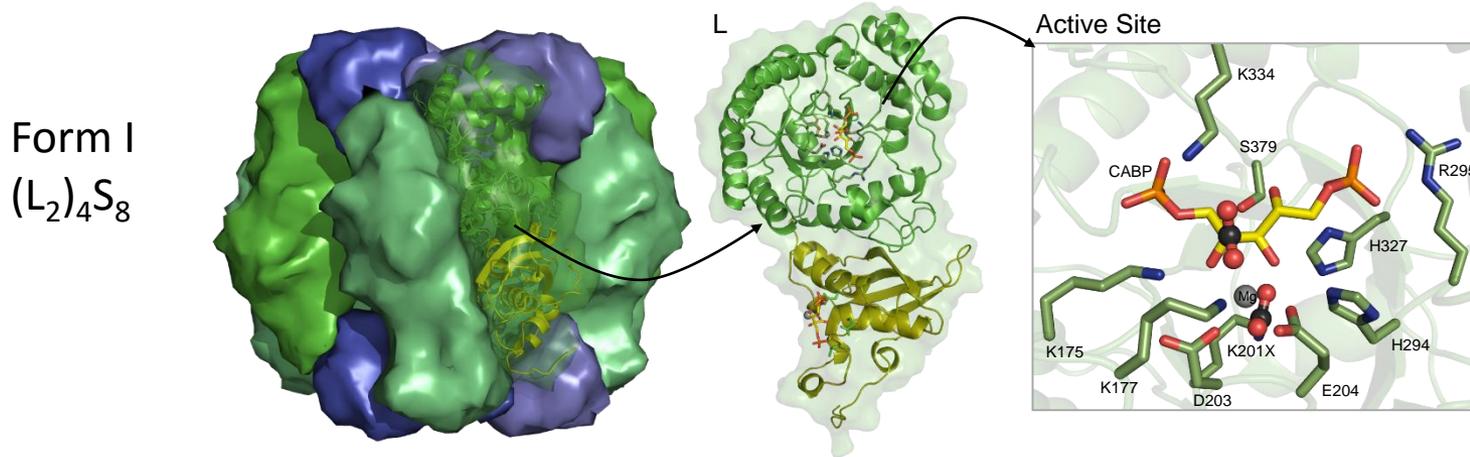
$K_m(O_2)$ (μM) : Rubisco affinity for O_2 .

$S_{c/o} = \frac{V_c / K_c}{V_o / K_o}$: Rubisco specificity for CO_2 as opposed to O_2 .



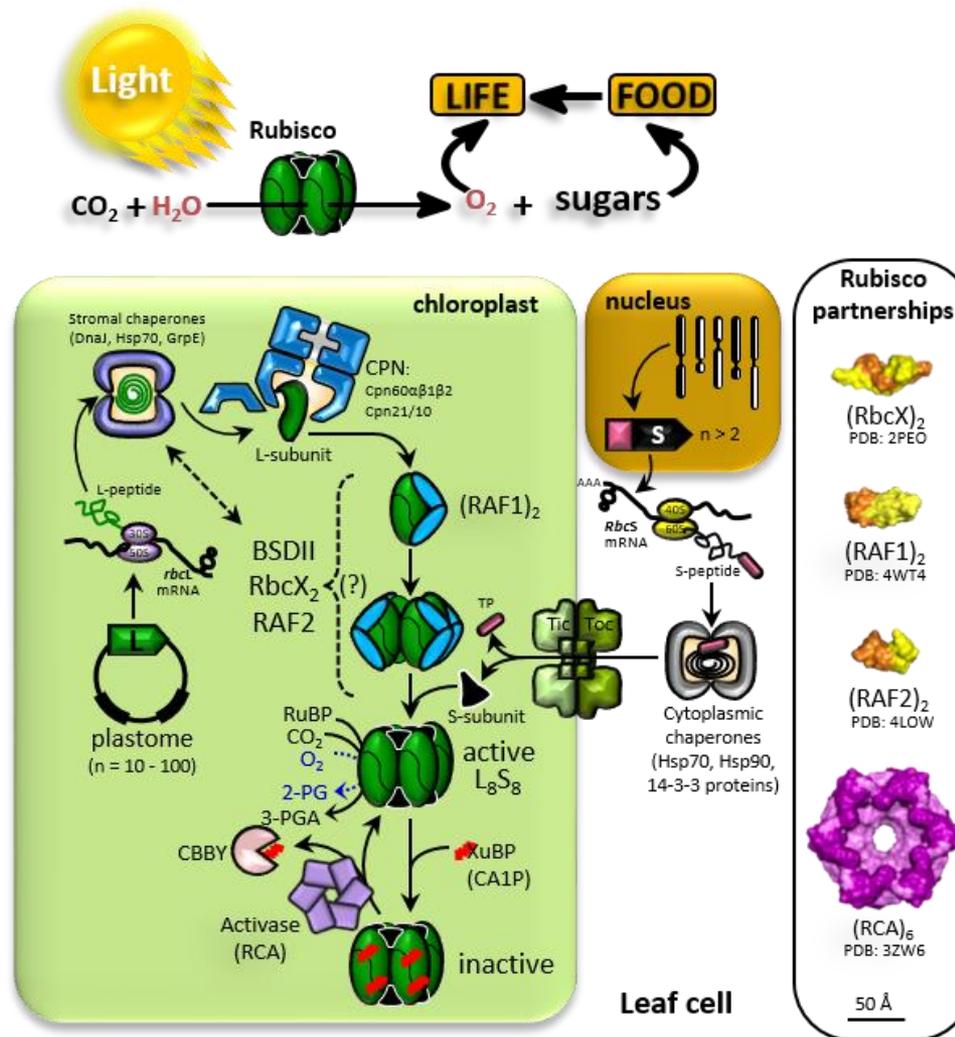
Nature holds the key to improved Rubisco

Whitney et al., (2011) Plant Phys.



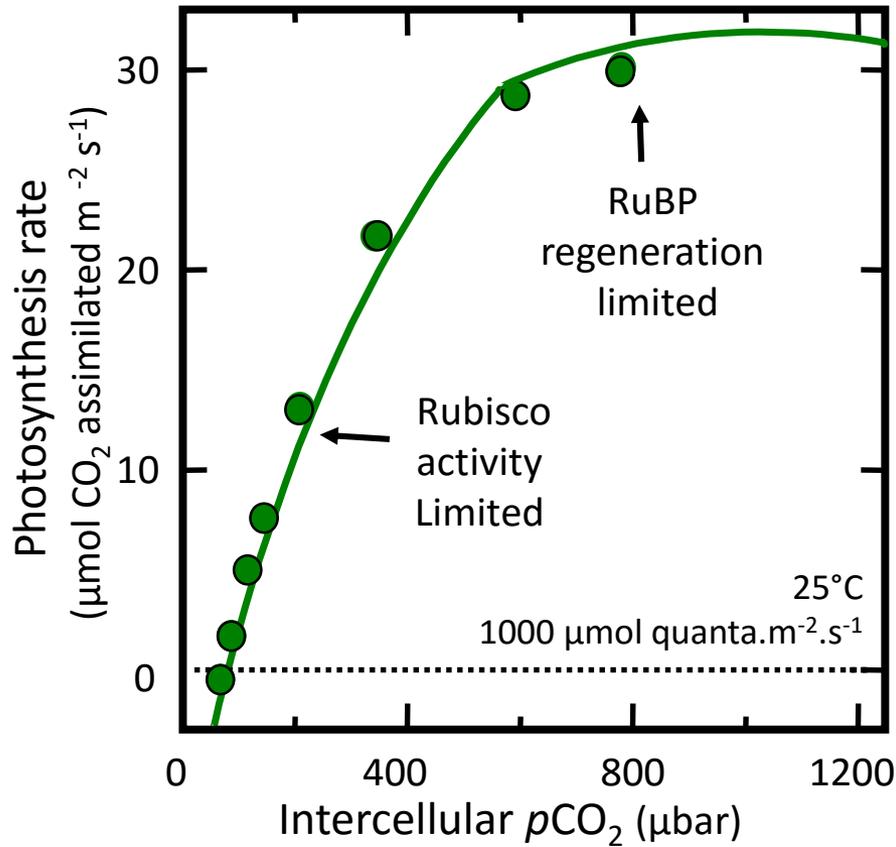
Form I		proteobacteria, cyanobacteria		algae		vascular plants	
Form II		chemoautotrophs proteobacteria		archaea		dinoflagellate algae	
Form III							

Rubisco assembly





Rubisco has a pervasive influence over photosynthesis



	V_c^{max} (s^{-1})	$S_{c/o}$	K_c^{air} (μM)	[Rubisco] ($\mu\text{mole} \cdot \text{m}^{-2}$)
tobacco	3.4	82.2	19.8	23

Natural Diversity in Rubisco catalytic properties may provide new prospects for improving CO₂ assimilation

Whitney et al., (2001) *Plant J.*

Griffithsia monilis

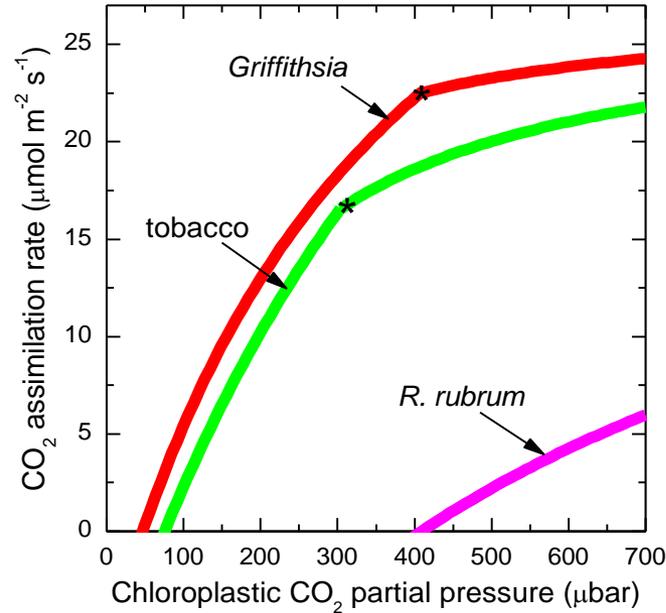
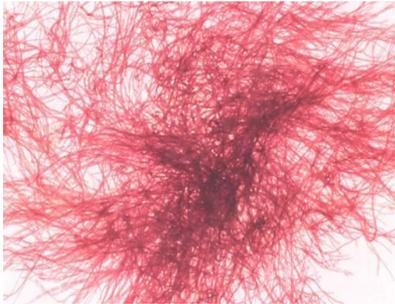


Table 1. Comparison of Rubisco kinetics

	Rubisco source		
	[†] tobacco	<i>Griffithsia</i>	<i>R.rubrum</i>
V_c^{max} (s ⁻¹)	3.4	2.6	5.8
* $K_m(\text{CO}_2)$ (µM)	19.8	12.6	28
** $S_{c/o}$	81	167	82

• Measured at 21% O₂

•• CO₂/O₂ specificity measured according to ref. 4.

Modelled CO₂ assimilation responses of Rubisco enzymes within a C3 plant.

CO₂-assimilation modelled according to von Caemmerer, (2000) using the kinetic parameters of each enzyme listed in Table 1.

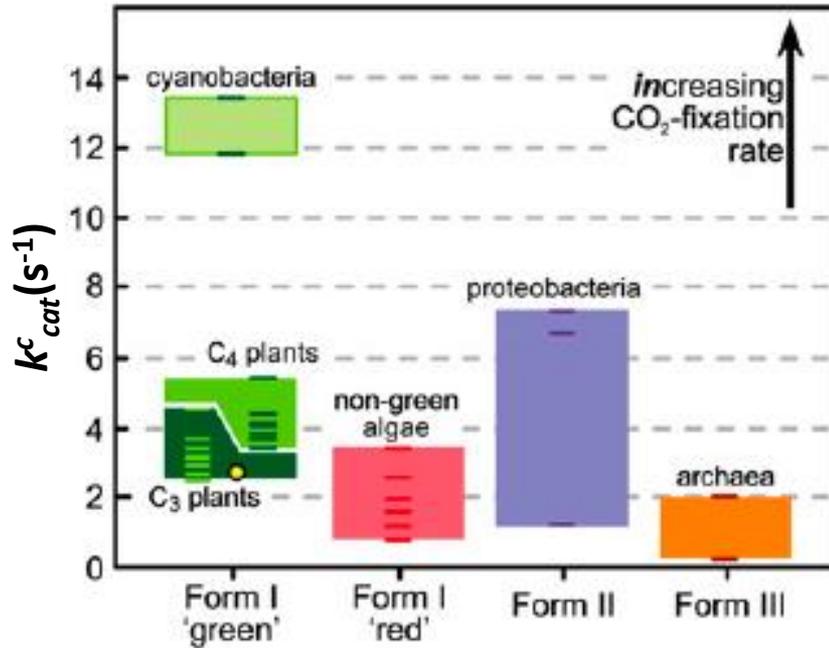
Modelling assumed:

Electron transport rate (J) to be 120 µmol.m⁻².s⁻¹, Rd - 1 µmol.m⁻².s⁻¹ and Rubisco sites- 20 µmol.m⁻².s⁻¹.

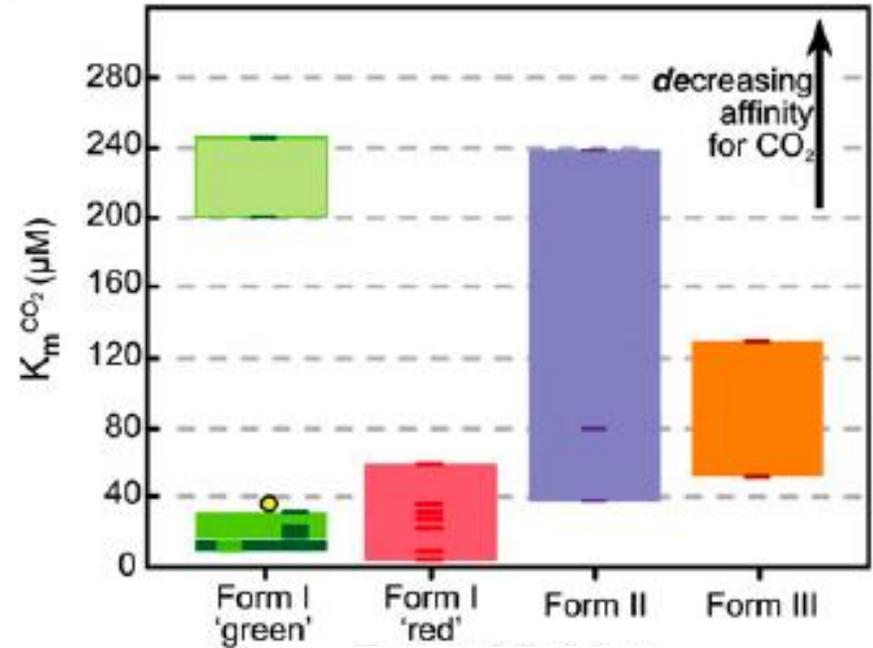
*Represents transition from Rubisco limited assimilation rate to light-limited regeneration of RuBP assimilation rate.

Nature holds the key to improved Rubisco

Whitney et al., (2011) *Plant Phys.*



Types of Rubisco



Types of Rubisco

Rubisco catalytic properties change with photosynthetic biochemistry within C₃, C₃-C₄, and C₄ Flaveria

Flaveria pringlei (C₃)



F. floridana (C₃-C₄)

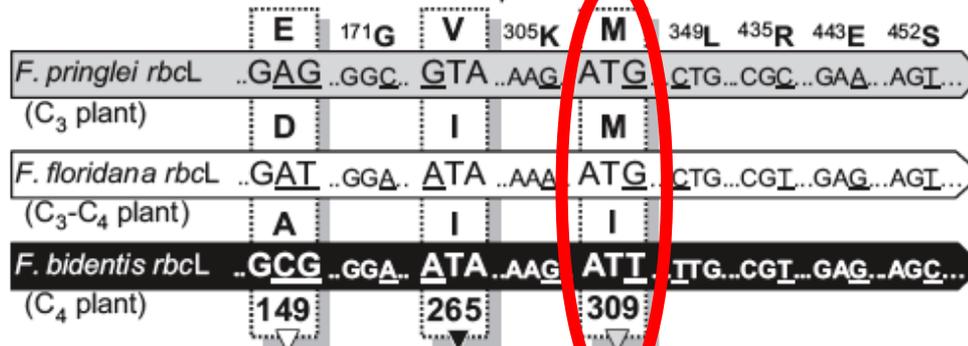


F. bidentis (C₄)



Plant	K_{cat}^c (s ⁻¹)	$K_c^{21\%O_2}$ (μ M)	$S_{c/o}$	$K_{cat}^c/K_c^{21\%O_2}$ (mM.s ⁻¹)
tobacco	3.2	22	82	130
<i>F. pringlei</i> (C ₃)	3.1	17	81	190
<i>F. floridana</i> (C ₃ -C ₄)	3.2	18	84	180
<i>F. bidentis</i> (C ₄)	4.2	28	76	150

Flaveria Rubisco L-subunit sequence variation



This residue is also C₄ catalytic switch for Neurachne.

Hudson *etal.* (1990) *JBC* **265**: 808-814

Kubien *etal.* (2008) *J Ex Bot.* **59**: 1767-1777

Whitney, S.M., Sharwood, R.E., et al., (2011) *PNAS*

Structure function relationships used to tailor Rubisco to future climates

- Identify L_{Su} catalytic switches that transfer C₄ - like kinetics to C₃ Rubisco.
- With the knowledge gained from temperature dependence of Rubisco catalysis design enzymes better adapted to extreme climates.
- Harness new technology to investigate if catalytic switches occur within Rubisco S_{Su}'s (Elena Martin).



Improve C₃ Crop Rubiscos

Translational applications of this Research to Australia's cotton industry

- Native to the Americas, Africa, India, Australia
- Grown in warm/hot environments
- Perennial plant grown as an annual
- Intensively managed crop
 - Fertiliser
 - 80% irrigated
 - 99% transgenic (insect & herbicide resistance)
- Notable industry outputs (last 20 years)
 - Australian growers 2 ½ times global average yield
 - Decrease insecticide use by 87%
 - Increase WUE by 40%



Courtesy: Nicola Cottee



Australian Government
Cotton Research and
Development Corporation



Pilot study funded by the CRDC to unravel the biochemistry underpinning heat tolerance and WUE.

DP – DP16: old
S71 – Sicot 71: new
L23 – SIOKRA L23: WUE
50 – CS50: Reduced WUE
64 – 64224-212: Thermotolerant
V2 – Sicala V-2 Poor thermotolerance.

Cotton Growth temp - 28°C



Cotton Growth temp 32°C



Comparison of cotton plants grown under elevated temperature



DP S71 L23 50
Old New + -
WUE



DP S71 L23 50
Old New + -
Thermo



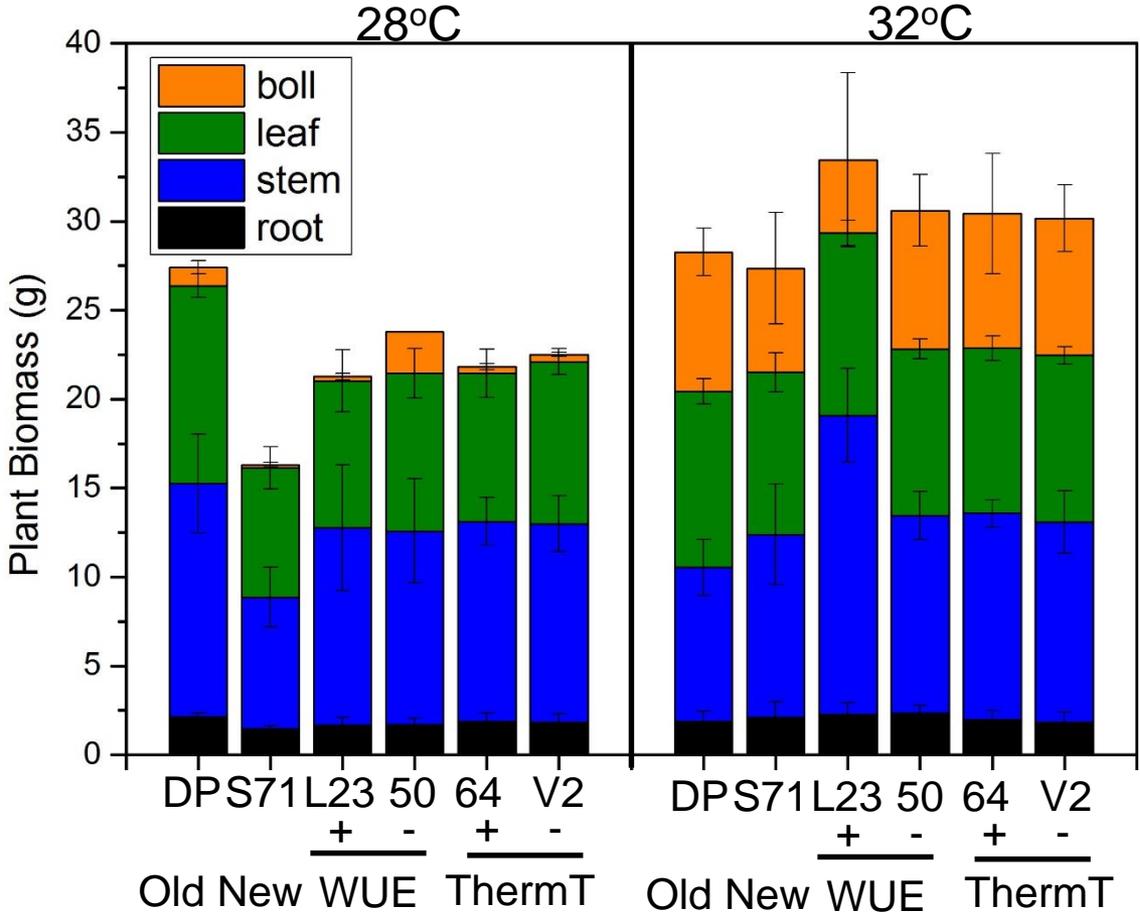
DP S71 L23 50
Old New + -
WUE



DP S71 L23 50
Old New + -
Thermo

92 DAP

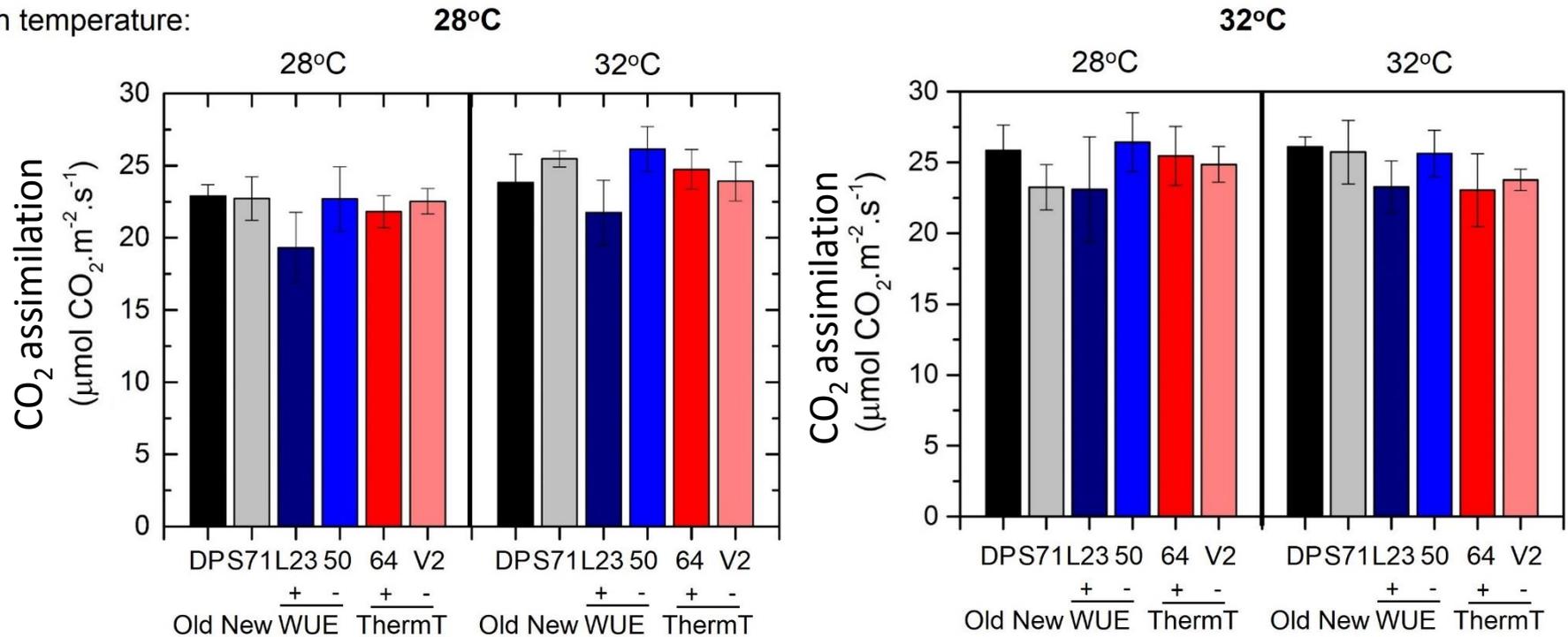
Total biomass measurements for cotton genotypes grown at 28°C and 32°C



Harvested 92 DAP.

Photosynthetic carbon assimilation at 28 and 32°C.

Growth temperature:

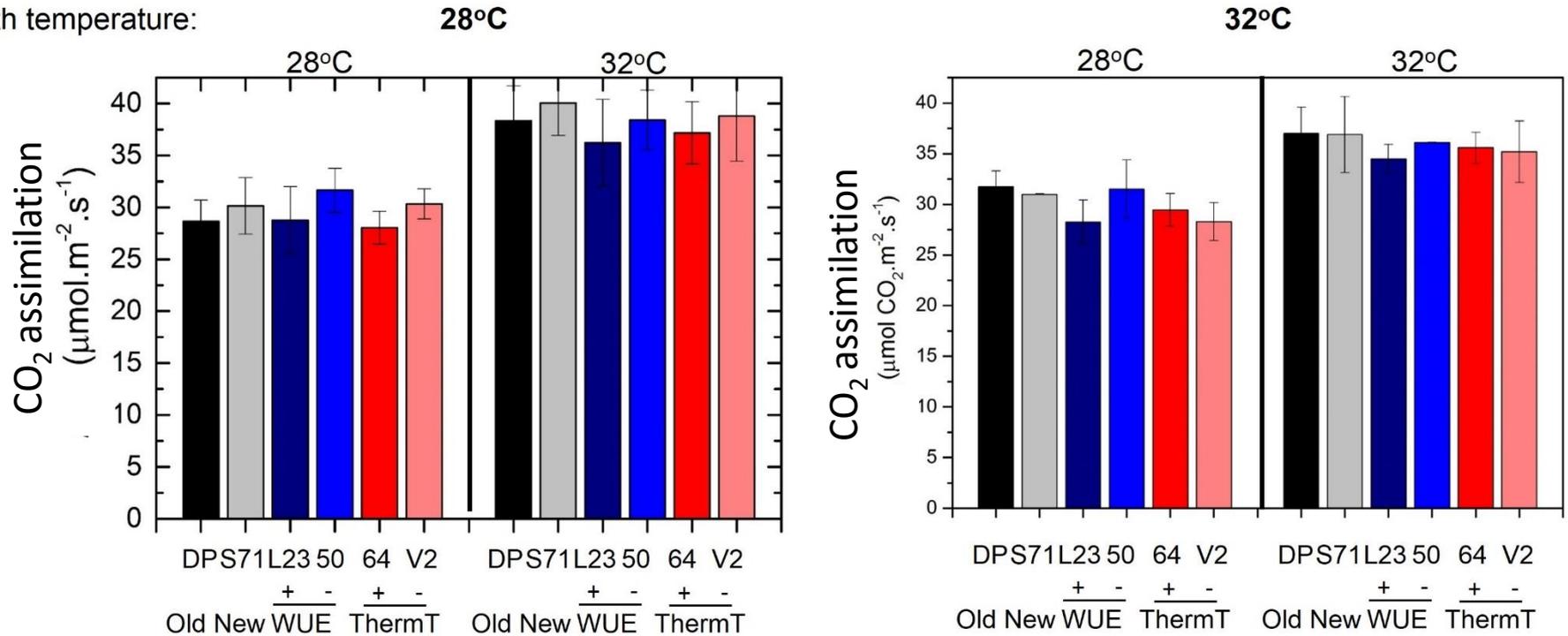


PAR: 1800 μmol.m⁻².s⁻¹

CO₂: 400 μbar

Photosynthetic capacity increased with temperature.

Growth temperature:



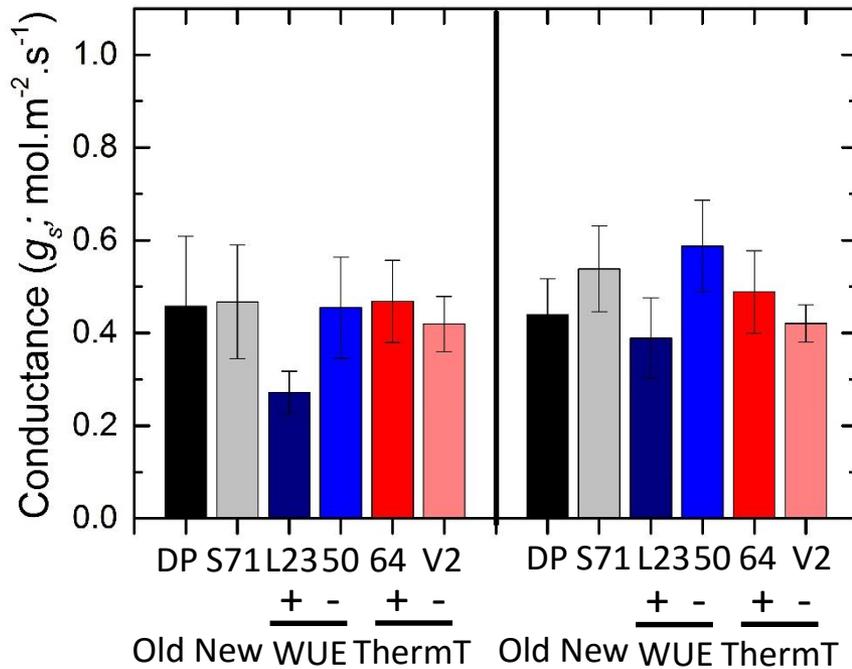
PAR: $1800 \mu\text{mol.m}^{-2}.\text{s}^{-1}$

CO₂: 1800 μbar

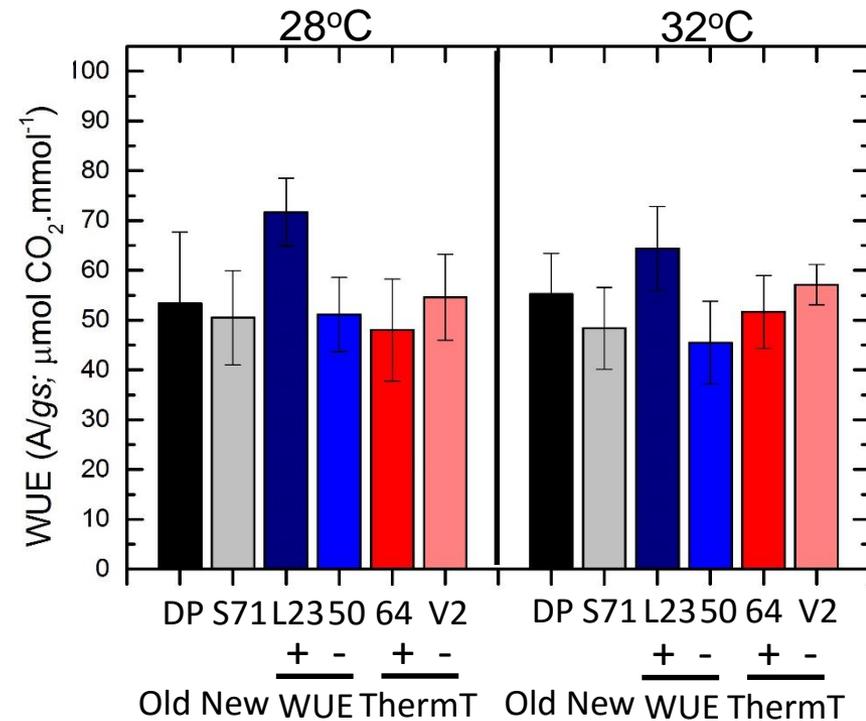
Genotype influences WUE_i

Plants grown at 28°C

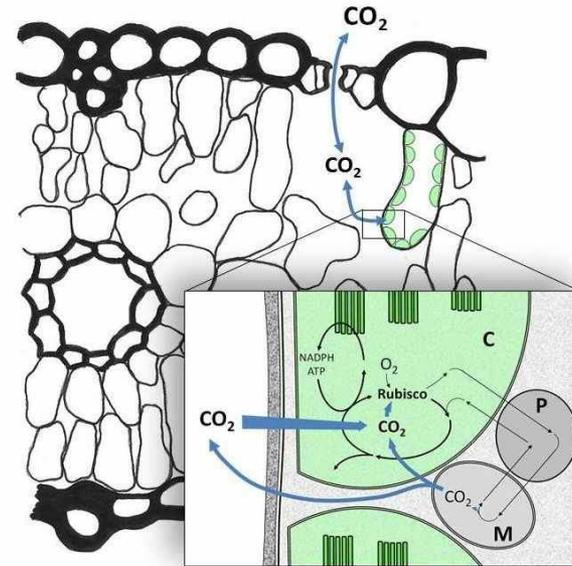
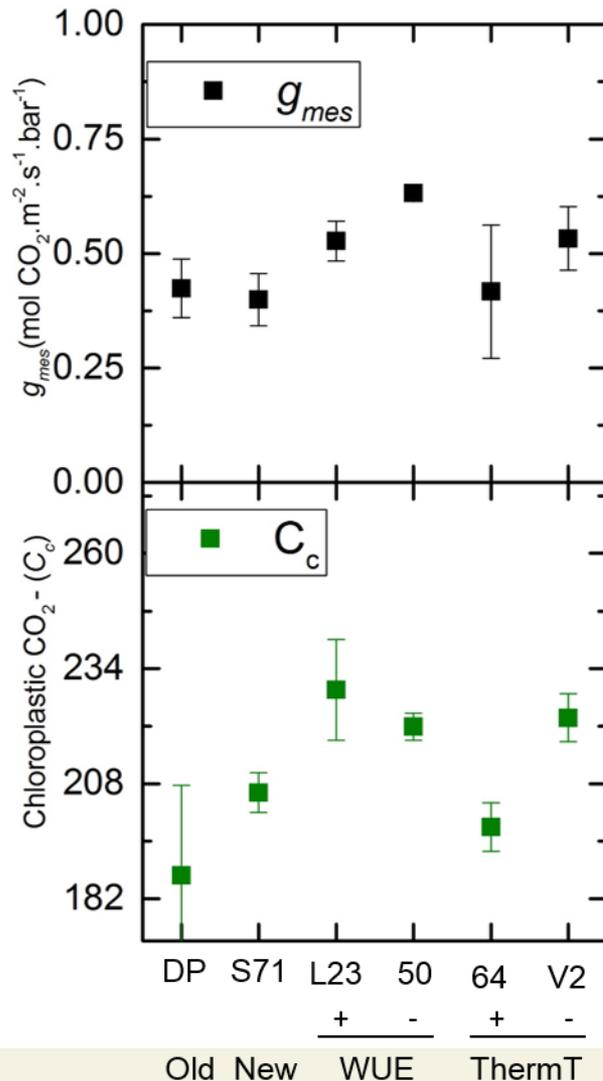
Measurement temp: 28°C



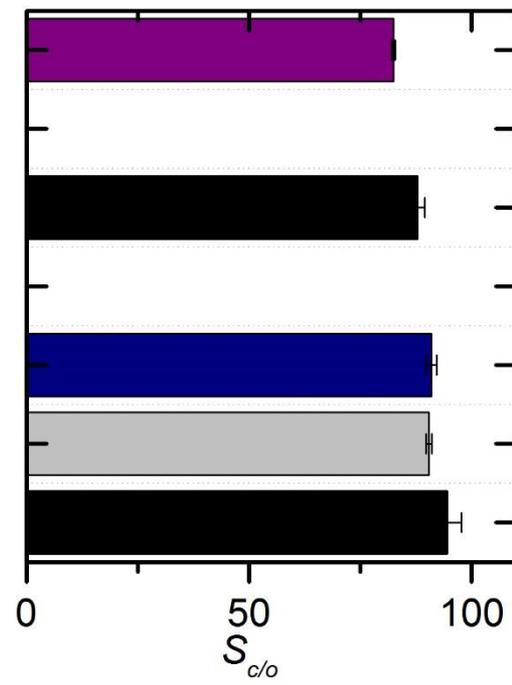
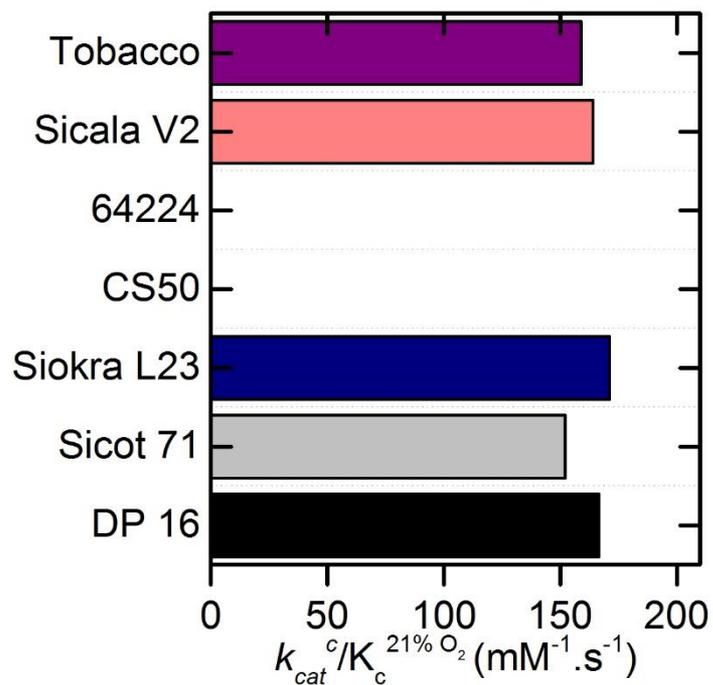
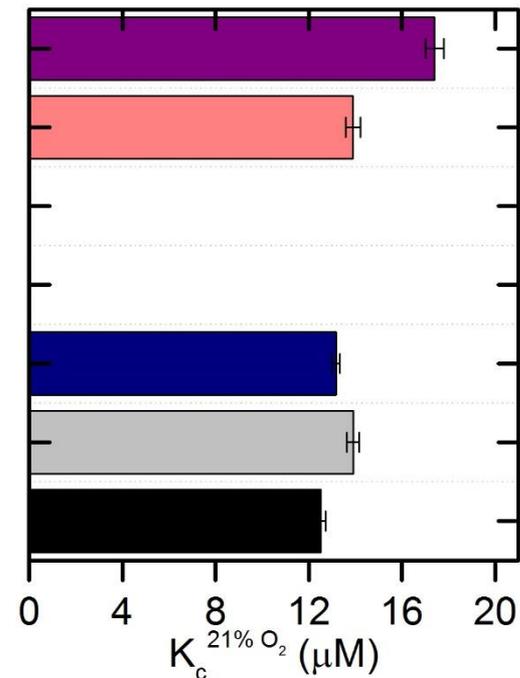
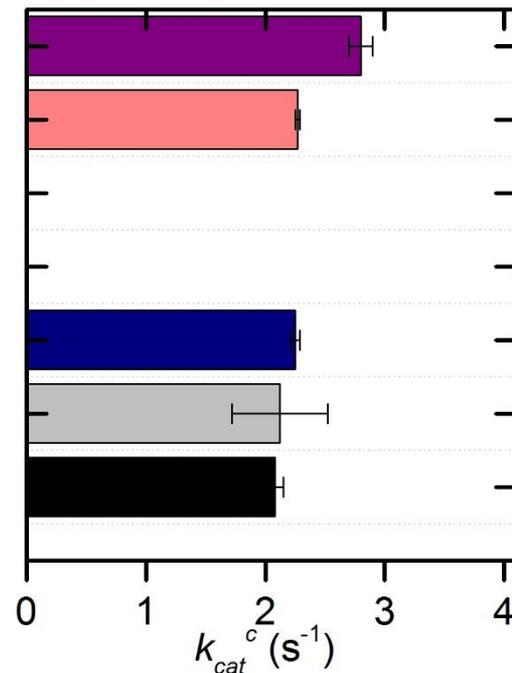
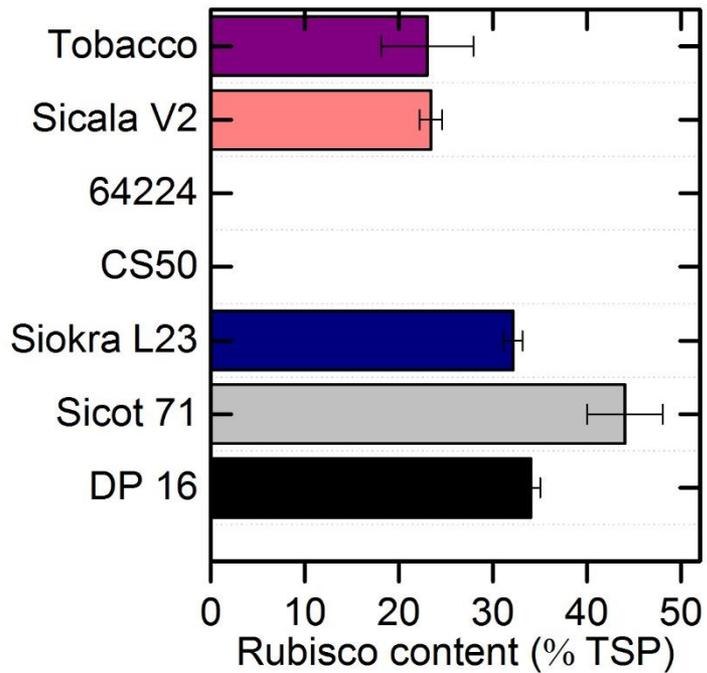
32°C



Genotype influences mesophyll conductance and chloroplastic CO₂



$$g_m = \frac{\left(b - a_i - \frac{eR_d}{A + R_d}\right) \times \frac{A}{p_a}}{a + \frac{(b - a)p_i}{p_a} - \Delta - \frac{eR_d(p_i - \Gamma^*)}{(A + R_d)p_a} - \frac{f\Gamma^*}{p_a}}$$



Comparison of modelled CO₂ assimilation for tobacco and cotton based on *in vitro* Rubisco kinetics

Rubisco kinetic property

In vitro measurements

Tobacco

Cotton

$K_C^{21\%O_2}$ (μM)

17.4 (521 μbar)

12.5 (374 μbar)

$S_{C/O}$

82.2

94.6

V_C^{max} (s^{-1})

2.8

2.08

$V_C^{max} / K_C^{21\%O_2}$ ($\text{s}^{-1} \text{mM}^{-1}$)

160.1

166.4

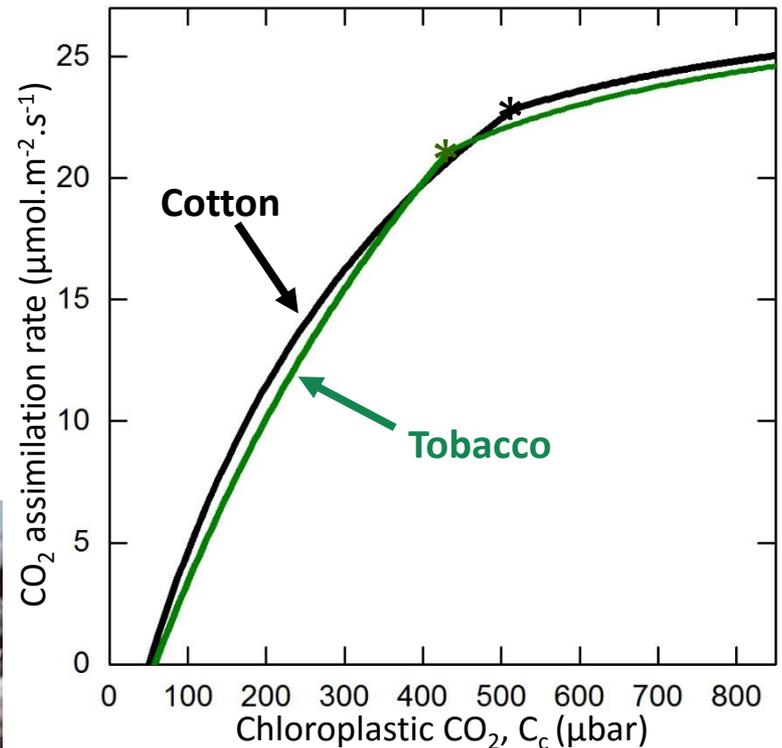


Tobacco



Cotton

Modelled CO₂ assimilation



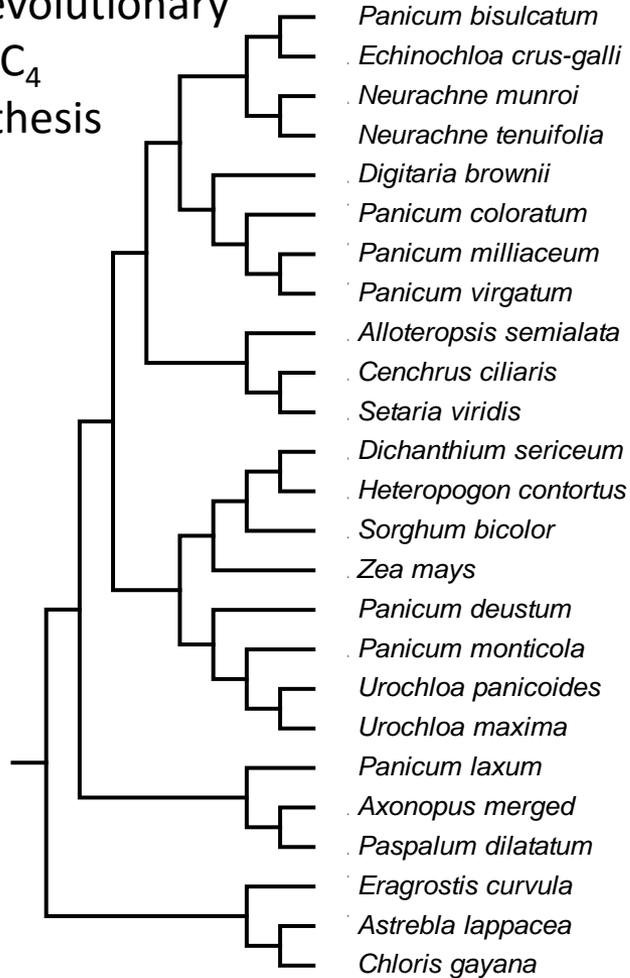
$J=120\mu\text{mol.m}^{-2}.\text{s}^{-1}$

Rubisco Sites= $20\mu\text{mol.m}^{-2}$

Rd= $1\mu\text{mol.m}^{-2}.\text{s}^{-1}$

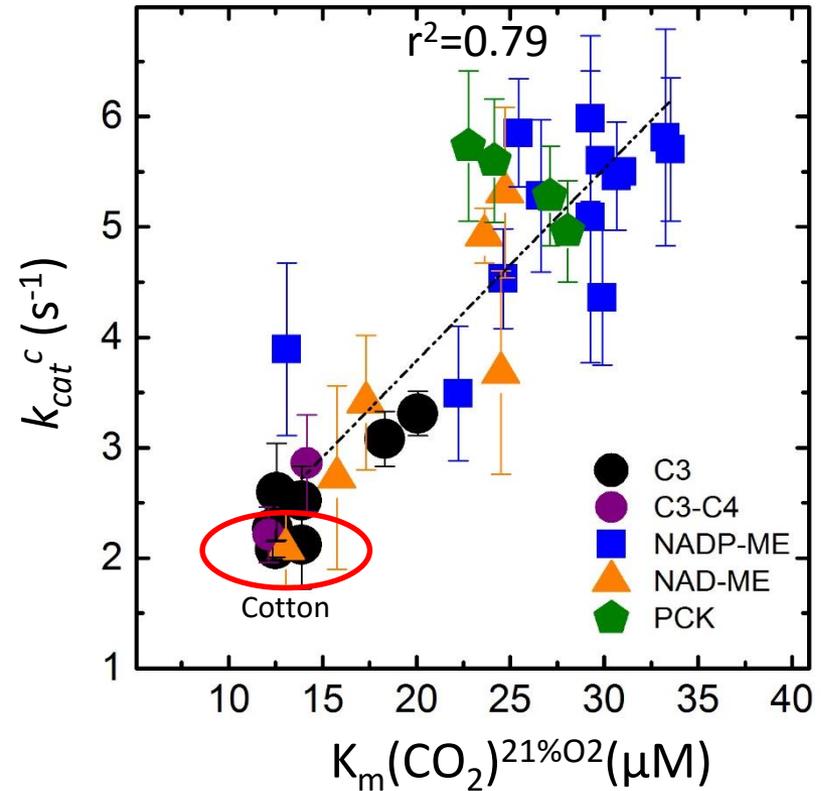
Diversity of Rubisco kinetics measured within C₄ grasses provide the path to improve cotton CO₂ fixation.

Multiple evolutionary origins of C₄ photosynthesis

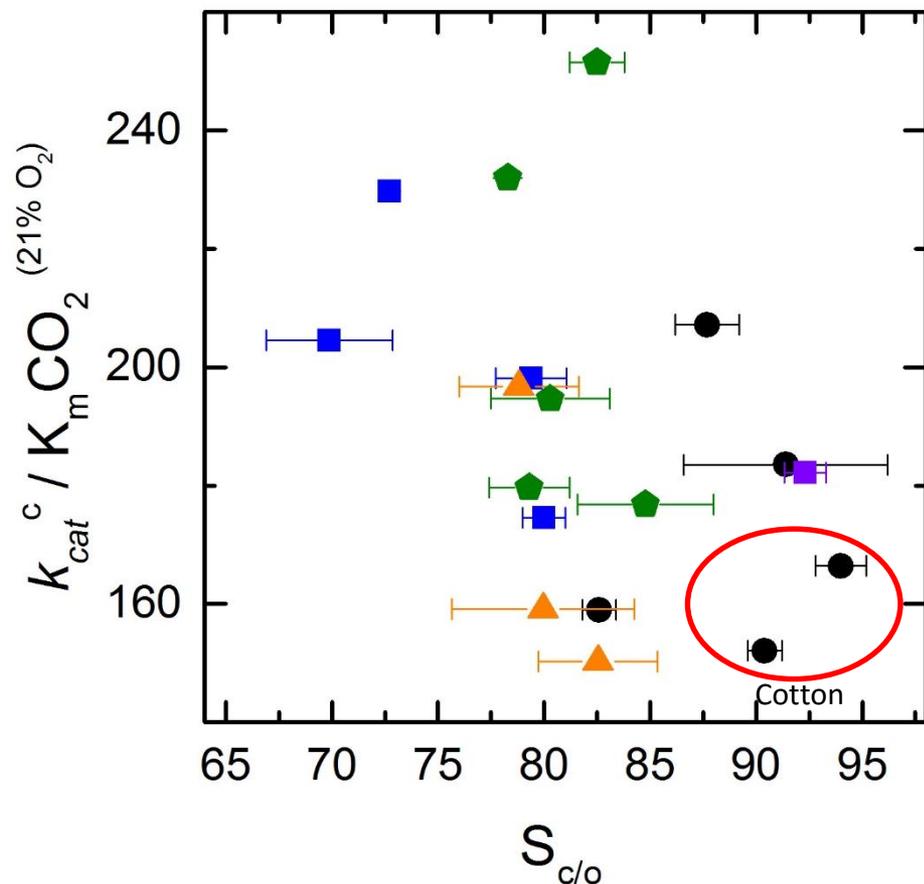
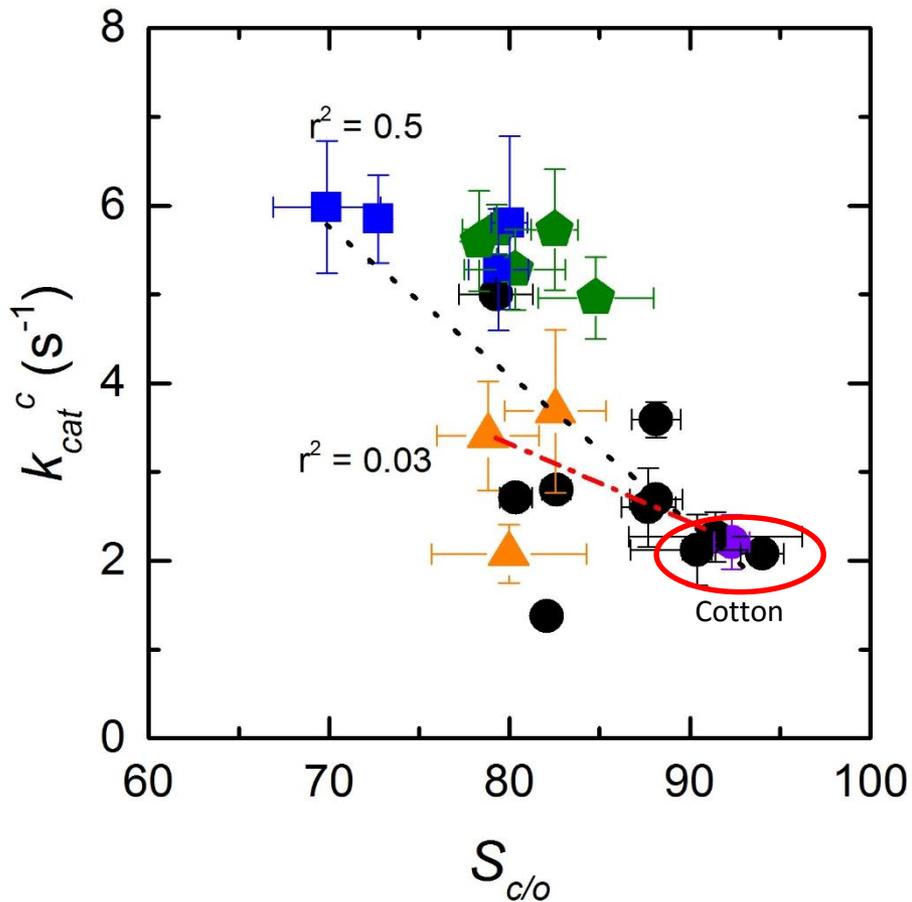


(Maxim Kapralov)

Sharwood, Whitney and Ghannoum



Improving cotton Rubisco catalysis



Chloroplasts have their own genome:

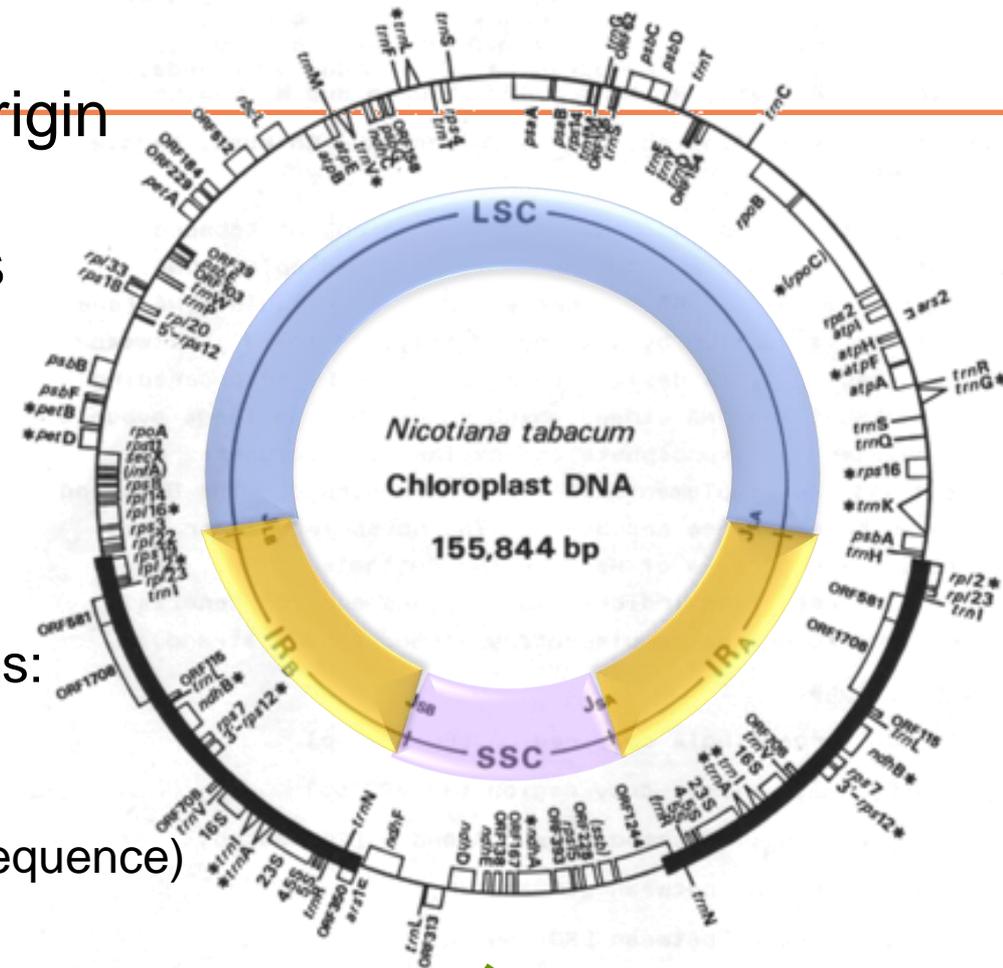
- prokaryote endosymbiotic origin
- semiautonomous organelles (nuclear dictatorship)
- circular chromosome-**plastome** (Largely prokaryotic like)

Plastomes are divided into 4 regions:

1x large single copy (LSC)

1x small single copy (SSC)

2x inverted repeat (IR) (duplicated sequence)



- different types of plastids

proplastid - amyloplast - leucoplast -
etioplast - chloroplast - chromoplast



Chloroplasts have their own genome:

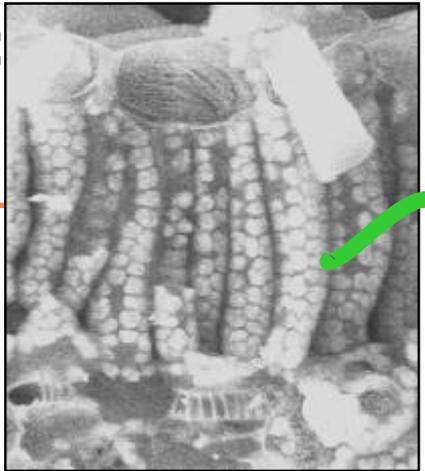
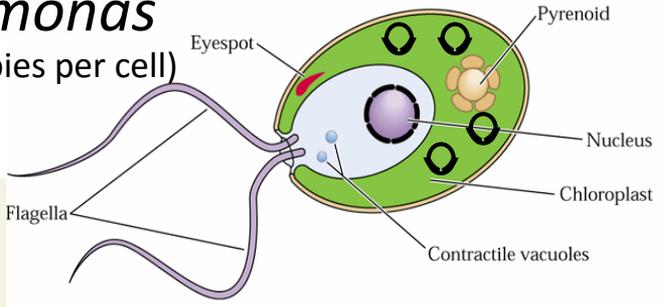
- high genome copy number
Each cell can have ~100 chloroplasts, each with ~100 plastome copies. Can thus have ~10,000 plastome copies/cell

- protein factories of the cell
Rubisco constitutes ~20-50% of leaf soluble protein

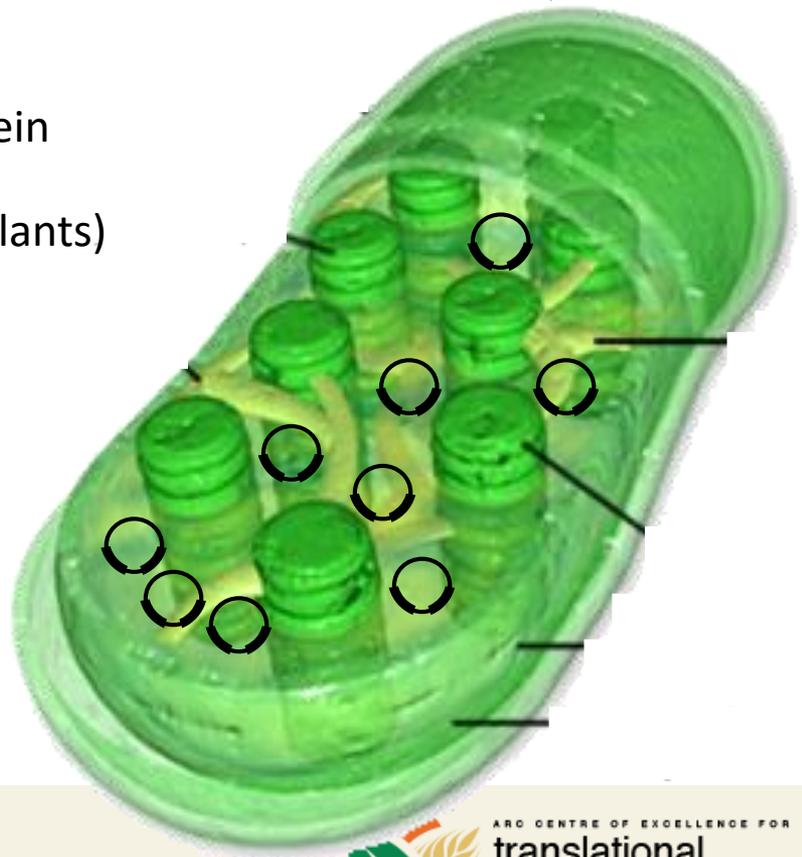
- Plastome maternally inherited (In most plants)
Plastome reproduction by binary fission
ie. independent of cell division

- Plastome readily transformable in some plants and the unicellular alga

Chlamydomonas (~80 plastome copies per cell)



tobacco leaf cross section



Requirements for stable transformation of a plastome

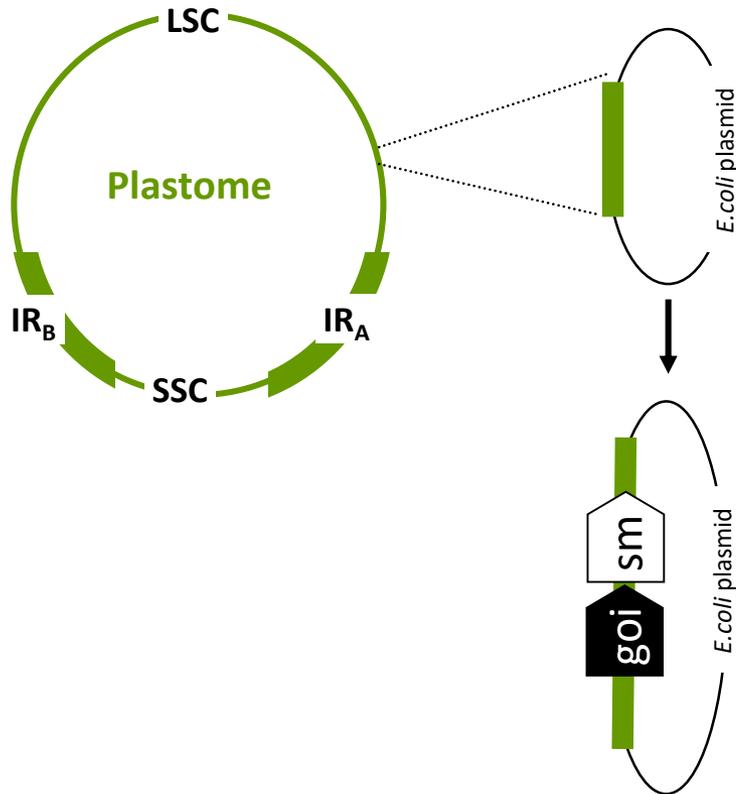
1. construct a transforming DNA coding the required genetic changes flanked by part of the plastome sequence
(allows for homologous recombination)
2. introduce the transforming DNA into the plant cells
3. selectively eliminate non-transformed plastome copies

Segregate until **HOMOPLASMIC**.

(*ie.* the transplastomic plant contains a pure population of only transformed plastome copies).

Requirements for stable transformation of a plastome:

1. Making a plastome transforming plasmid



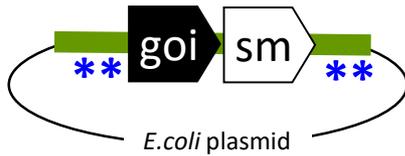
(Step 1) Sub-clone the area of plastome you wish to modify into an *E. coli* plasmid

(Step 2) Make the desired genetic modifications (e.g. insert a gene of interest (goi) along with a selectable marker gene (sm))

The *aadA* gene is the common selectable marker gene used that confers resistance to the antibiotic spectinomycin which inhibits protein synthesis

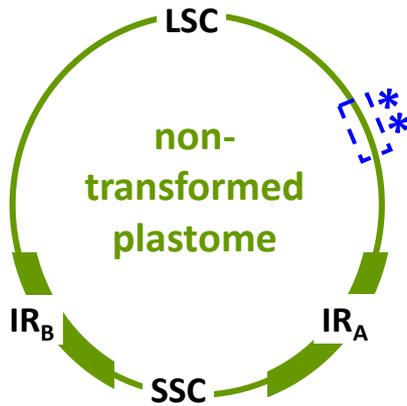
Requirements for stable transformation of a plastome:

1. How does the DNA integrate into the plastome?

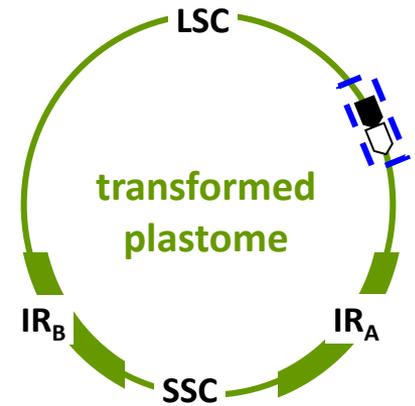


Transformation
(process of introducing
recombinant DNA into
the host organism)

- Homologous recombination: a process of *directed insertion* of recombinant DNA into a genome.



the regions of plastome flanking sequence in the transforming plasmid (**) DNA direct the integration of the genetic changes into the target region of the plastome via homologous recombination



Requirements for stable transformation of a plastome:

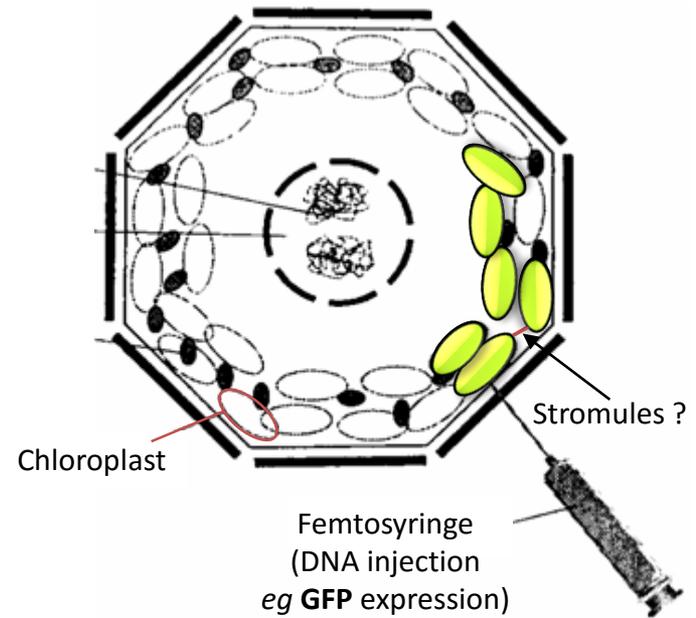
2. introducing the transforming DNA into the cells

1. Polyethylene Glycol-

Requires a protoplast cell suspension, labour intensive, transformation is inefficient, prone to nuclear mutations. It is cheap though

2. Microinjection- femtosyringe injection of DNA directly into a chloroplast

- Genetic material transfer between chloroplasts through transient linkages called stromules (?)
- **Transient expression** only.



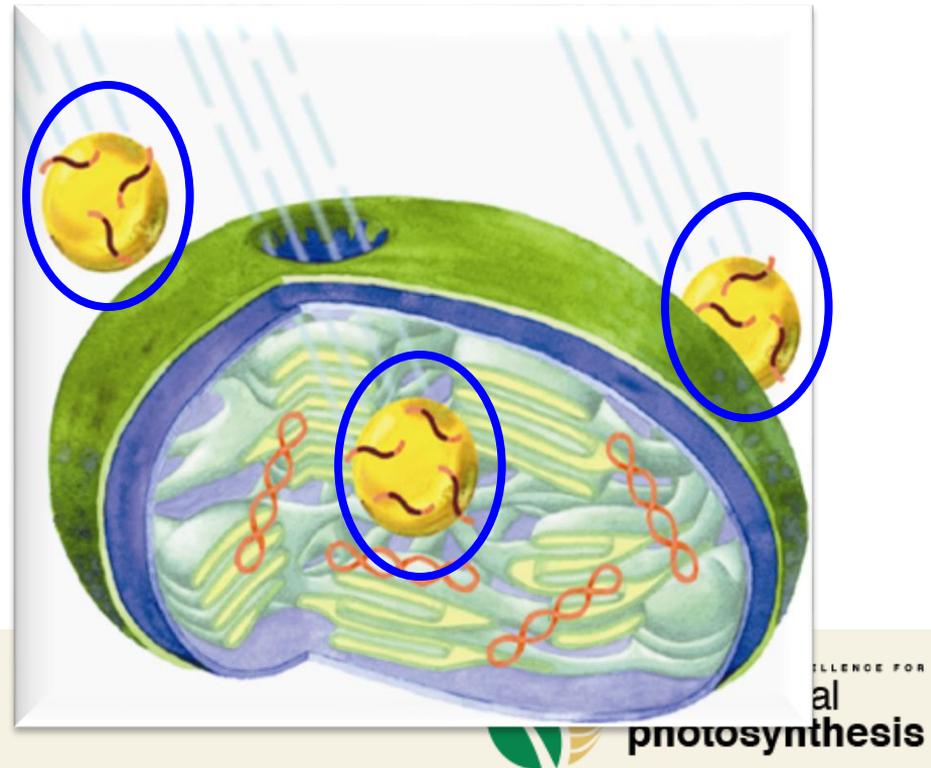
Bel, *et al.*, Current Opinion in Biotechnology (2001). **12**, 144-149

Requirements for stable transformation of a plastome:

2. introducing the transforming DNA into the cells

3. Particle bombardment-

- Directly bombard leaf tissue with transforming DNA coated onto an inert support (eg tungsten or gold particles)
- Rapid regeneration of transformed tissue
- Transformation efficiency is high for tobacco (model plant)
- Relatively expensive

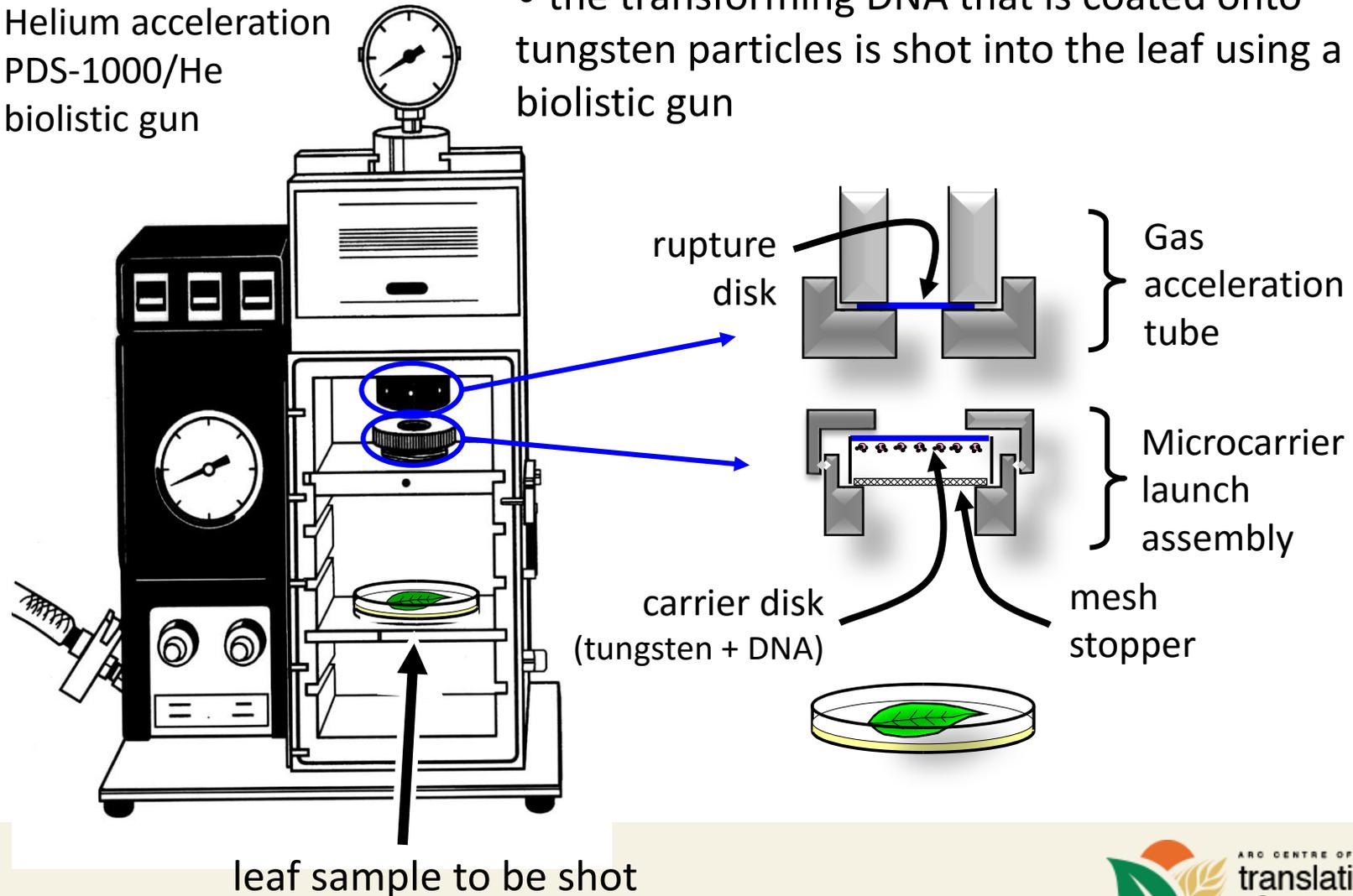


Requirements for stable transformation of a plastome:

2. introducing the transforming DNA into the cells

Helium acceleration
PDS-1000/He
biolistic gun

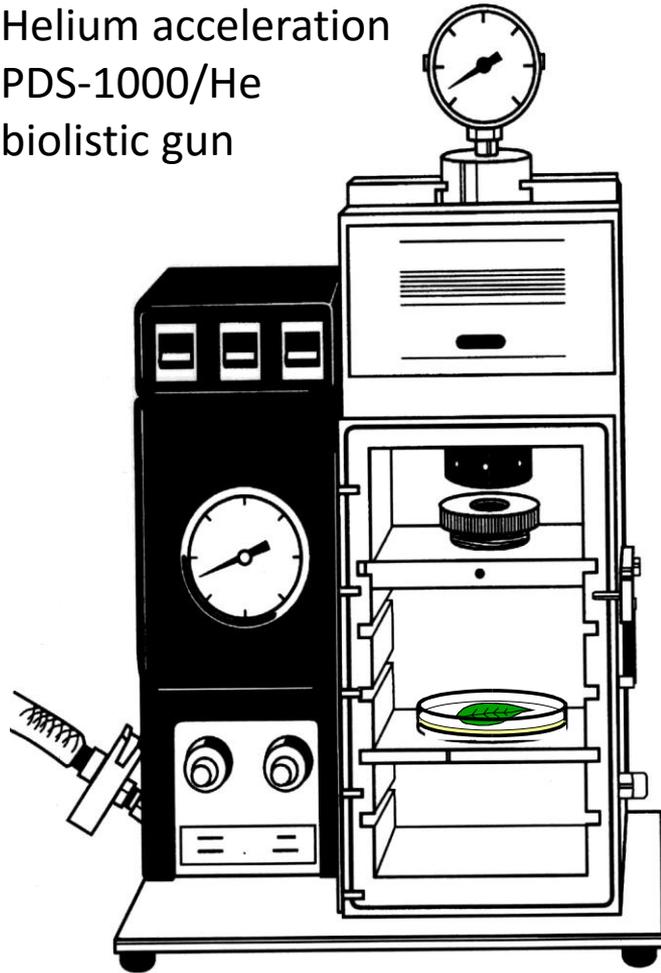
- the transforming DNA that is coated onto tungsten particles is shot into the leaf using a biolistic gun



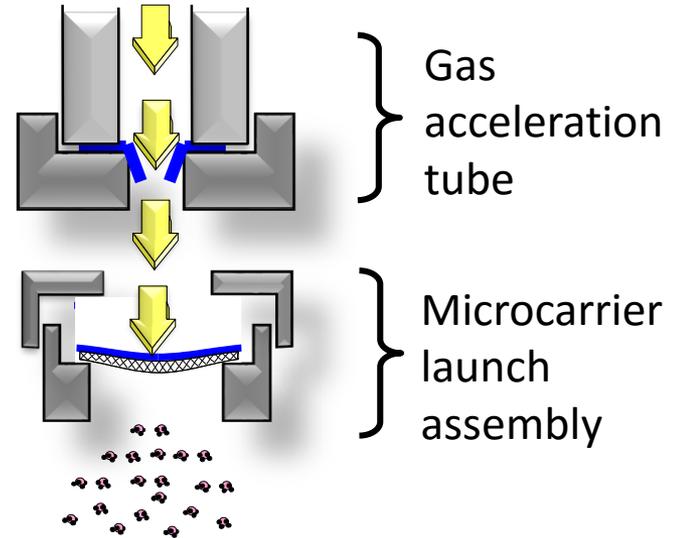
Requirements for stable transformation of a plastome:

2. introducing the transforming DNA into the cells

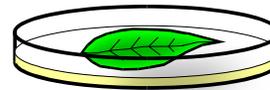
Helium acceleration
PDS-1000/He
biolistic gun



apply helium
pressure to gas



Regenerate tissue
and select for
transformants



Requirements for stable transformation of a plastome:

3. How do we eliminate non-transformed plastome copies?

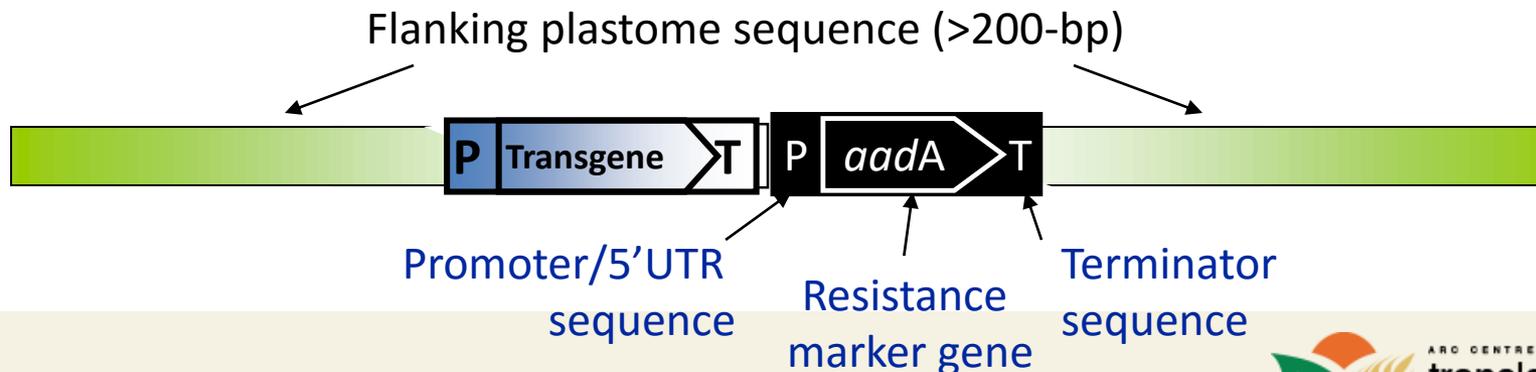
- use drug resistance marker selection to select for transformed plastome copies until tissue is **homoplasmic**.

(i.e. where there are no longer any wild-type, non-transformed, plastome copies)

- the *aadA* gene most common drug resistance marker used in plastid transformation

The *aadA* encodes an adenylyltransferase protein that confers resistance to the antibiotics spectinomycin and streptomycin. These antibiotics function to inhibit protein synthesis by binding to prokaryotic-like ribosomes and preventing translation.

GENERAL TRANSFORMING PLASMID DESIGN-



Requirements for stable transformation of a plastome:

3. How to select for homoplasmic plastome transformants

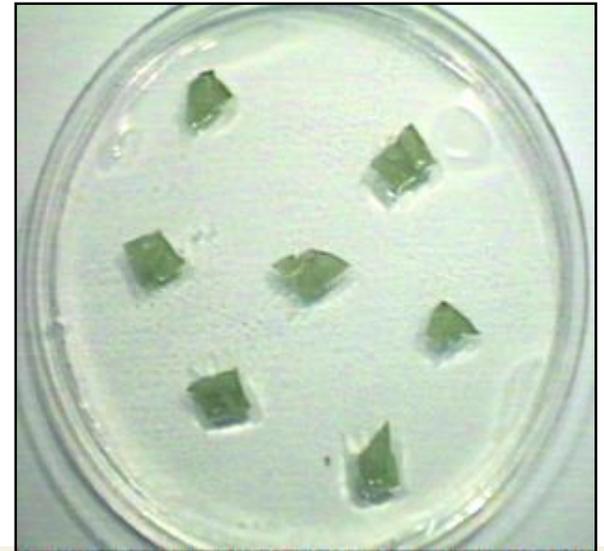
Leaf section prior to bombardment. Abaxial (underside) facing upwards



Two days post-bombardment. Leaf surface shiny where bombarded with DNA-coated tungsten



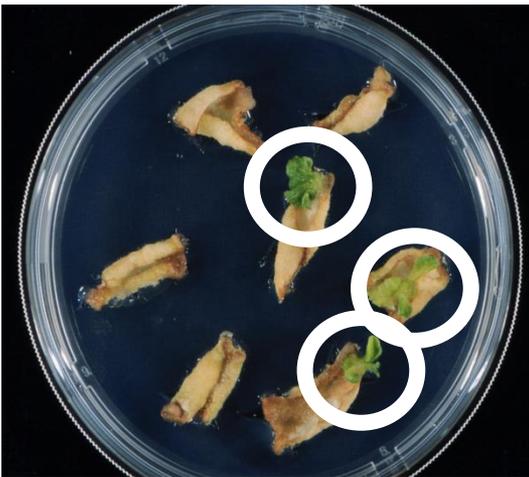
The leaf is now cut into $\sim 0.5\text{cm}^2$ sections and placed on tissue culture media containing spectinomycin ($0.5\text{mg}\cdot\text{ml}^{-1}$)



Requirements for stable transformation of a plastome:

3. How to select for homoplasmic plastome transformants

After 3 to 7 weeks spectinomycin resistant transformants appear as green tissue.



The spectinomycin resistant tissue is transferred to fresh spectinomycin containing tissue culture media



And the transformed tissue allowed to regenerate (~4 weeks)



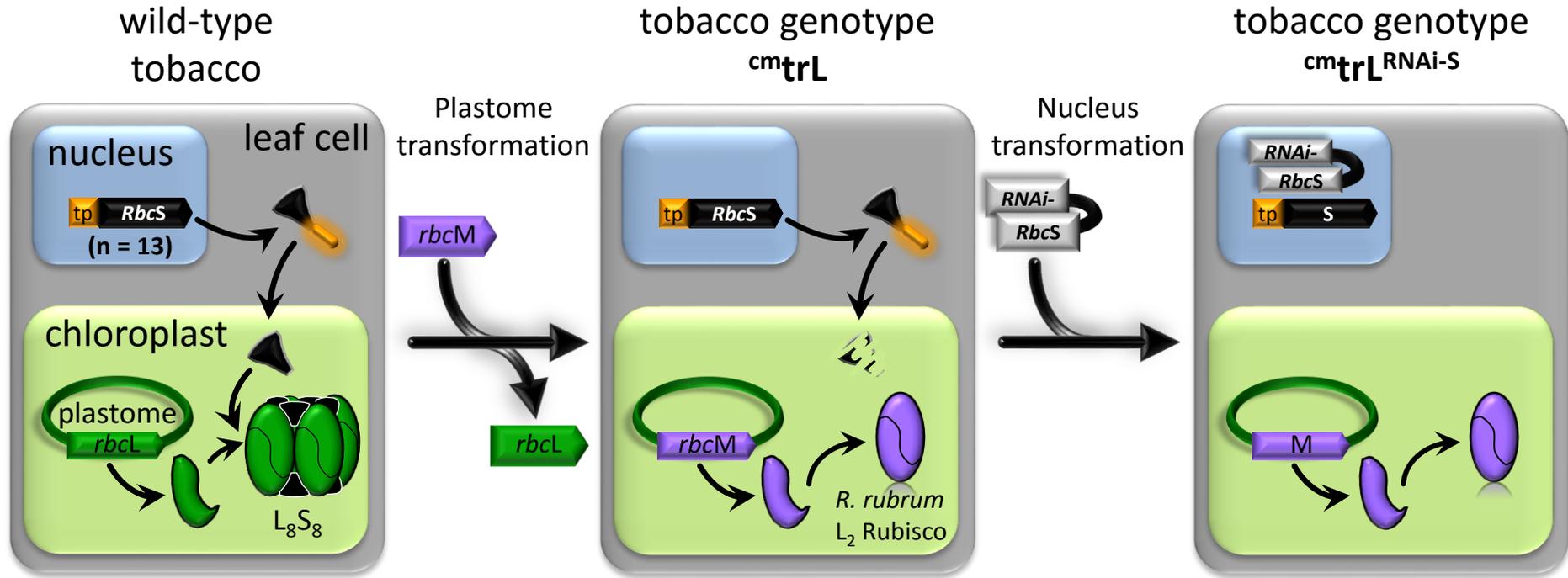
Selection/ regeneration process repeated 2 to 4 times until tissue homoplasmic

Step 1 ----->

Step 2 ----->



Tobacco genotypes used to study Rubisco in leaf chloroplasts



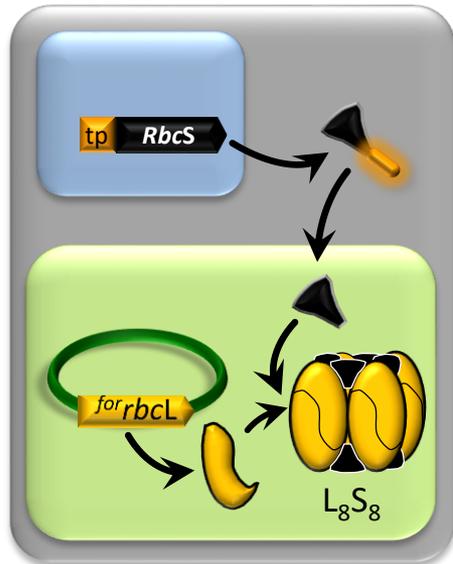
Whitney and Sharwood (2008)
J. Exp Botany. 59: 1909-1921

Yi-leen Lim (poster)



Hybrid Rubisco studies in leaf chloroplasts

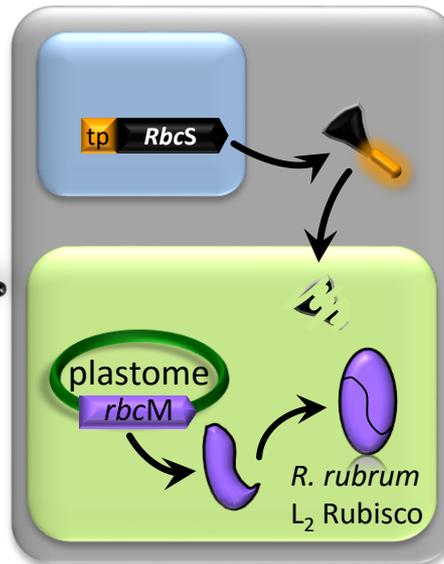
Hybrid Rubisco producing tobacco genotype



Plastome transformation



tobacco genotype ^{cm}trL



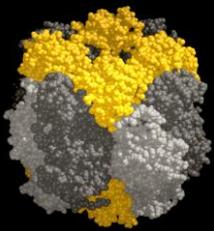
Plant L-subunits introduced

- Sunflower
- *Flaveria* sp. (C₃, C₄ & C₃-C₄)
- Arabidopsis

Sharwood et al. (2008) *Plant Physiology* 146: 89-96

Whitney et al., (2011) *PNAS* 108: 14688-93

Whitney et al., (2015) *PNAS* In press



Acknowledgements



University of
Western Sydney

HAWKESBURY INSTITUTE
FOR THE ENVIRONMENT

David Tissue

Oula Ghannoum

Balasaheb Sonawane



Michael Bange

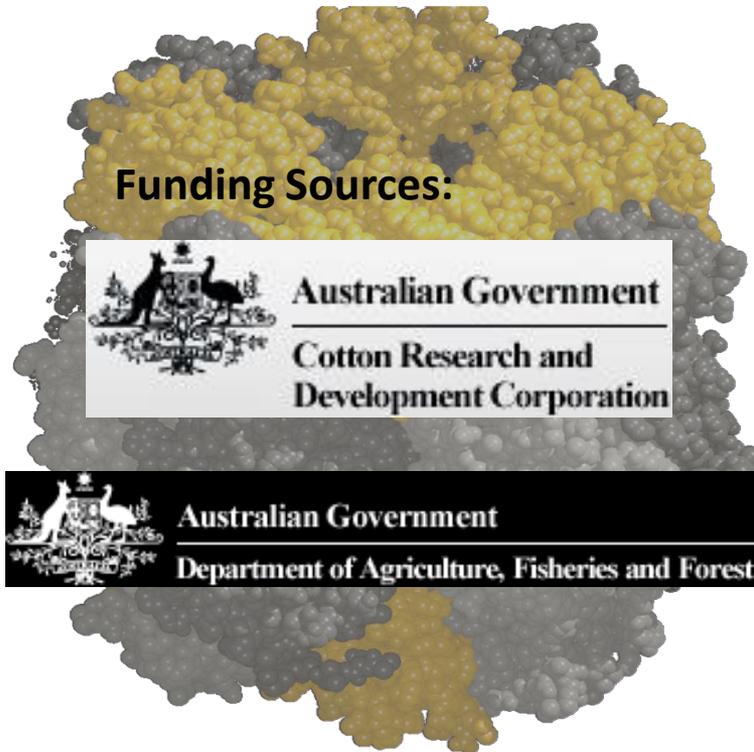
Nicola Cottee

Warren Conaty



Boyce Thompson Institute
for Plant Research

David Stern



Funding Sources:



Australian Government

Cotton Research and
Development Corporation



Australian Government

Department of Agriculture, Fisheries and Forestry



ANU

THE AUSTRALIAN NATIONAL UNIVERSITY

Spencer Whitney

Susanne von Caemmerer

Bob Furbank

Maxim Kapralov

Carly Conlan

Elena Martin Avila



Tom Brutnell

Tim Anderson



THE UNIVERSITY OF
WESTERN AUSTRALIA

Martha Ludwig

Ian Small



Robert E. Sharwood^{1,2}, Oula Ghannoum¹, Balasaheb Sonawane¹, Renee Smith¹, Spencer M. Whitney², Michael Bange³ and David Tissue¹.

Funded Research by:



¹Hawkesbury Institute for the Environment, University of Western Sydney, Richmond 2753, NSW, Australia.

²Research School of Biology, Australian National University, Canberra 0200, ACT, Australia.

³CSIRO Plant Industry, Narrabri 2390, NSW, Australia.



1. Research Background and Aims:

Australia's cotton industry is a world leader in production, generating some of the highest yields and quality fibre in the world. However, global climate change and associated problems with variable climate (drought and high ambient air temperature) impact crop productivity. One approach applied in the industry is to develop varieties that have greater WUE and are more heat tolerant. The CSIRO have identified varieties in commercial field conditions that are more WUE and heat tolerant and have developed crop and plant physiological measurements to phenotype these varieties. Notwithstanding, we still lack an understanding of the detailed physiological and biochemical attributes conferring greater WUE and heat tolerance and the project framework adopted to address these gaps is outlined in figure 1.

The aims of this research are to:

- 1) Improve our understanding of the underlying physiology of photosynthesis that contribute to differences in WUE and heat tolerance to allow breeders to focus on traits that deliver the greatest benefits.
- 2) Investigate the biochemistry underpinning improvements in heat tolerance and water-use to enable cotton researchers to make more informed choices about selecting lines that are better suited to current and future climates; and
- 3) Interrogate cotton Rubisco catalytic properties and assess the possibility for improvement in CO₂ fixation.

Cotton varieties that are heat-tolerant, water use efficient, together with old and new varieties were grown in the UWS glasshouse under current (28°C) and future projected (32°C) air temperatures. In a second phase the same genotypes were exposed to water deficit at both growth temperatures and identical measurements were made to identify varieties that are resilient to drought and increased ambient air temperatures.

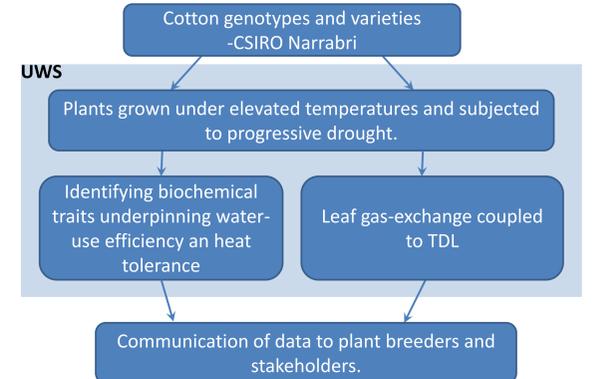


Cotton Growth temperature: 28°C

Cotton lines used in this study.

DP – DP16: old.
S71 – Sicot 71: Industry standard.
L23 – SIOKRA L23: Improved WUE.
50 – CS50: Reduced WUE.
64 – 64224-212: Thermotolerant.
V2 – Sicala V-2: Poor thermotolerance.

Unravelling biochemical and physiological elements that underpin heat tolerance and water – use efficiency:



Phase 1 – Grow genotypes under non-limiting water and nitrogen conditions at 28 and 32°C. Determine the relationship of photosynthesis to the underlying biochemistry.
Phase 2 – Grow genotypes and expose them to drought conditions at 28 and 32°C. Determine the impact on photosynthesis for each genotype.

Figure 1. Project framework.

By adopting a multidisciplinary approach this project seeks to improve our understanding of the underlying physiology contributing to heat tolerance and water use efficiency in cotton.

2. Carbon assimilation increases with increasing growth temperature.

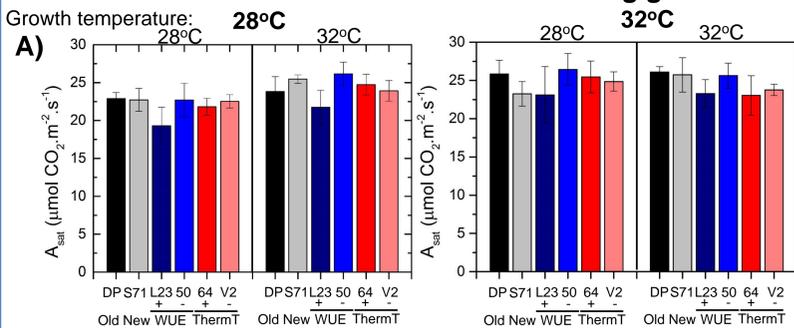
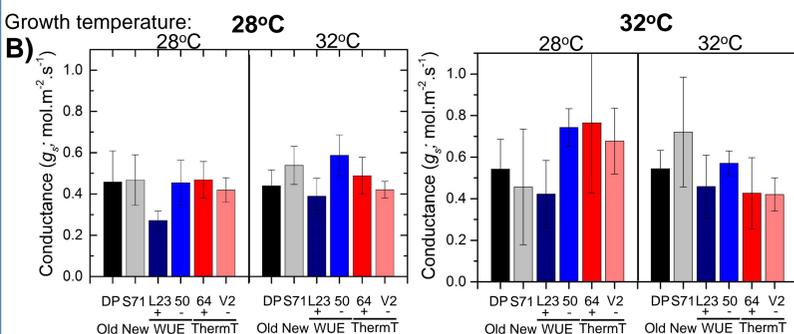


Figure 2. Measurement of light saturated CO₂ assimilation and stomatal conductance.

(A) Light saturated rates of CO₂ assimilation measured at 28 and 32°C for cotton genotypes grown at a daily average temperature of 28 and 32°C, respectively. (B) Stomatal conductance (g_s) determined for cotton genotypes as indicated above. PAR – 1800 μmol quanta m⁻² s⁻¹, CO₂ – 400 μbar.



3. Photosynthetic capacity increases with increasing ambient air temperature.

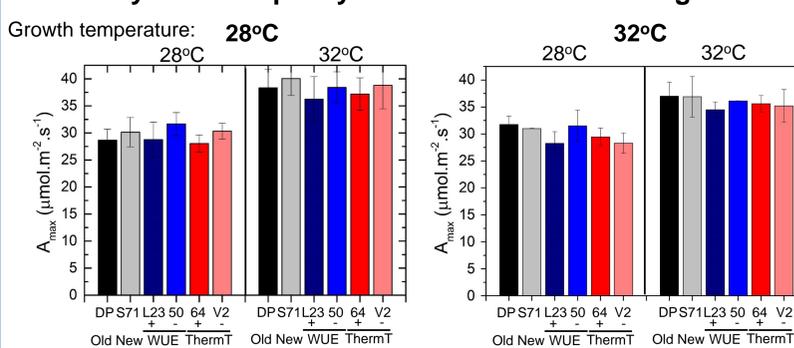


Figure 3. Measurements of maximum rates of CO₂ assimilation.

Maximum assimilation rates (A_{max}) measure for genotypes as outlined in figure 2. PAR – 1800 μmol quanta m⁻² s⁻¹, CO₂ – 1800 μbar.

4. Variations in leaf area, carbohydrates and biomass between cotton genotypes.

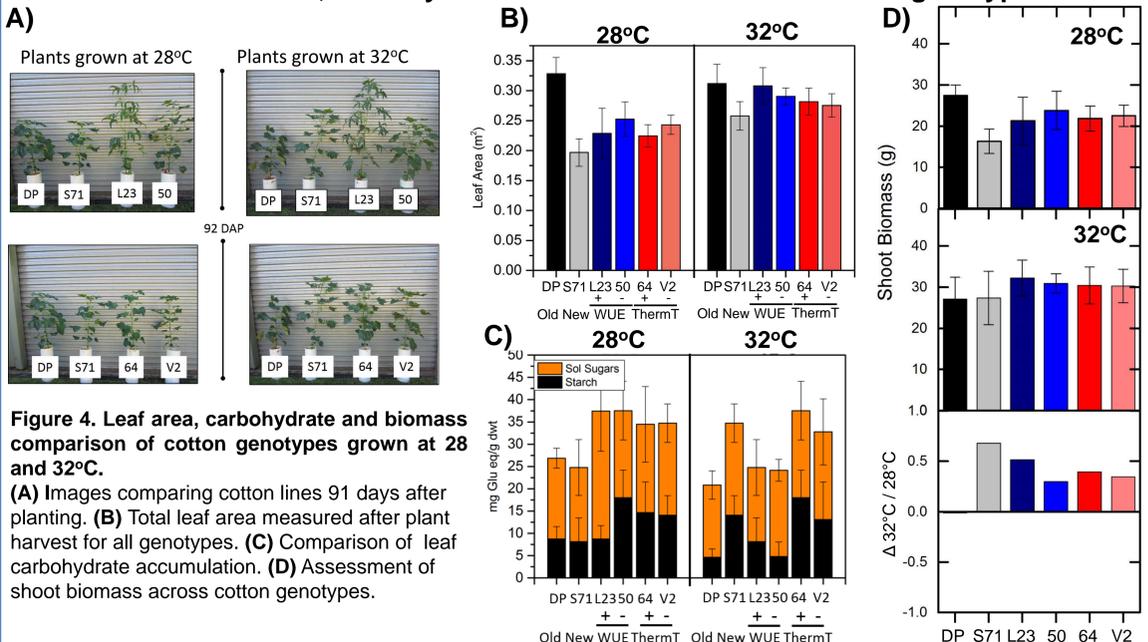


Figure 4. Leaf area, carbohydrate and biomass comparison of cotton genotypes grown at 28 and 32°C.

(A) Images comparing cotton lines 91 days after planting. (B) Total leaf area measured after plant harvest for all genotypes. (C) Comparison of leaf carbohydrate accumulation. (D) Assessment of shoot biomass across cotton genotypes.

5. Assessment of Cotton Rubisco content and catalytic properties.

- Significant investment of Nitrogen into Rubisco synthesis.
- Cotton Rubisco has a superior carboxylation efficiency and S_{c/o} compared to tobacco.

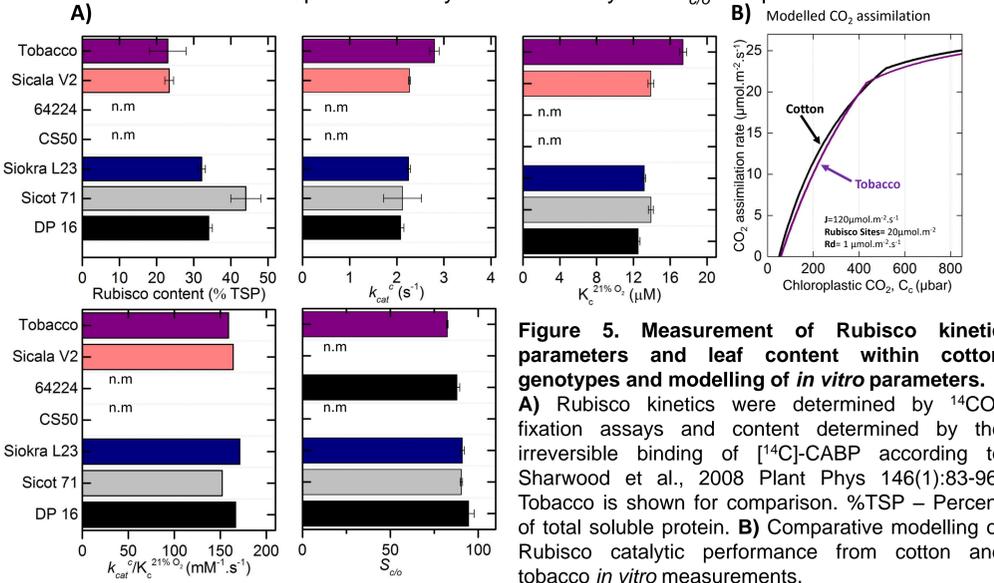


Figure 5. Measurement of Rubisco kinetic parameters and leaf content within cotton genotypes and modelling of *in vitro* parameters.

(A) Rubisco kinetics were determined by ¹⁴CO₂ fixation assays and content determined by the irreversible binding of [¹⁴C]-CABP according to Sharwood et al., 2008 Plant Phys 146(1):83-96. Tobacco is shown for comparison. %TSP – Percent of total soluble protein. (B) Comparative modelling of Rubisco catalytic performance from cotton and tobacco *in vitro* measurements.

6. Mesophyll conductance to CO₂ varies between genotype and under water deficit.

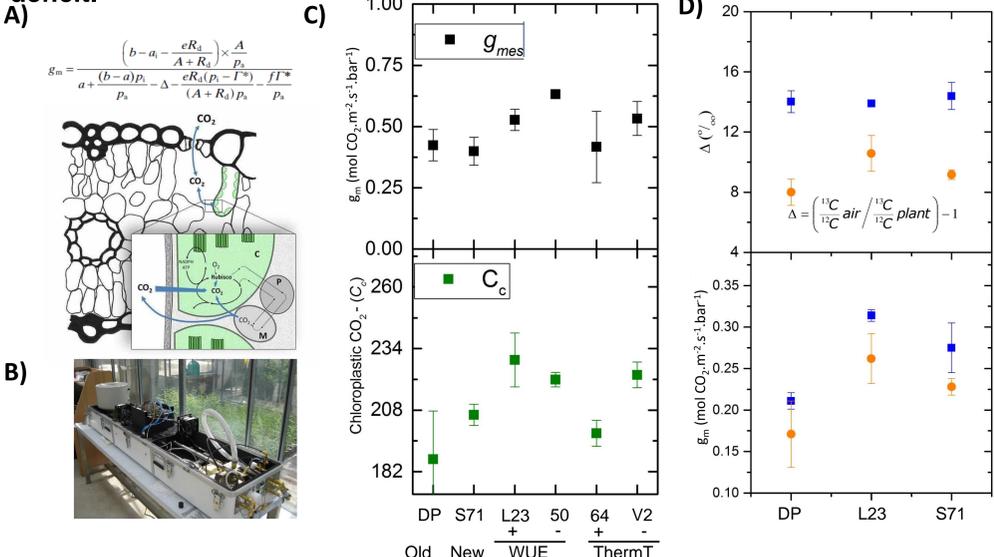


Figure 6. Measurement of mesophyll conductance by ¹³C/¹²C carbon isotope discrimination.

(A) Mesophyll conductance (g_{mes}) determined from gas-exchange and carbon isotope discrimination (Evans et al., 1986 AJPP 13: 281-292). Diagram from von Caemmerer and Evans, 2010 Plant Phys 154(2):589-92) showing the path of CO₂ into the chloroplast indicating the resistance to diffusion to the chloroplast. (B) Image of the TDL at UWS Richmond. (C) Comparison of g_{mes} among Cotton genotypes and the associated calculation of chloroplastic [CO₂] (C_c) (D) Calculation of photosynthetic ¹³C/¹²C discrimination (Δ) used to determine g_{mes}.

7. Conclusions:

- Increased temperature improved the photosynthetic capacity of all genotypes and development is accelerated with earlier onset of flowering and boll formation.
- Across genotypes there is variation in leaf area, plant biomass and carbohydrate content.
- Cotton plants invest large amounts of N into Rubisco synthesis providing scope for future improvements in NUE that would aid in reducing fertilizer application across the industry.
- Cotton Rubisco has improved catalytic performance compared to the tobacco counterpart and would offer a benefit of improved carbon fixation if transplanted into tobacco chloroplasts.
- Online ¹³C/¹²C discrimination measurements revealed variation in mesophyll conductance to CO₂ which impacted the chloroplastic CO₂ concentration. This suggests that plant breeding can enhance mesophyll conductance and should be a factor to screen for in future programs.