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Plastid Preview 2018, Lancaster University, Sept 3rd – Sept 4th

Monday September 3rd

13:00 – 13:50 Registration open (poster hanging)

13:50 – 14:00 Opening remarks by Elizabete Carmo-Silva, Lancaster University

Session 1 **Chair: Conor Simpson, University of Cambridge**

14:00 – 14:20 Lorna McAusland, University of Nottingham
High-throughput phenotypic exploration of photosynthetic traits; exploring novel genetic variation to promote high biomass and yield in wheat

14:20 – 14:40 Cristina Sales, Lancaster University
Potential photosynthesis is a key driver of yield in the Watkins wheat landrace collection

14:40 – 15:00 Rakesh Tiwari, University of Leeds
Leaf gas-exchange measurement with spectral capture to detect temperature-induced spectral signatures: a new method tested on soy and tropical trees

15:00 – 15:20 Alexandra Burgess, University of Nottingham
The fluctuating canopy light environment and implications for crop photosynthesis

15:20 – 16:00 Refreshment break (posters, key collection)

Session 2

16:00 – 16:20 Mary Williams, ASPB
Simple strategies to promote you and your science

16:20 – 16:40 Mike Whitfield, New Phytologist Trust
Promoting plant science and growing connections

16:40 – 18:00 Poster session, with refreshments

19:00 Bar opens, Barker House Farm

19:30 Conference dinner, Barker House Farm

Plastid Preview 2018, Lancaster University, Sept 3rd – Sept 4th

Tuesday September 4th

Session 3 **Chair: Nicky Atkinson, University of Edinburgh**

09:00 – 09:20 Monika Gajecka, University of Silesia in Katowice
Plastid fate at the initial stage of isolated microspore culture in spring barley

09:20 – 09:40 Jonathan Griffin, Lancaster University
Chloroplast RNA-binding proteins (cpRNP), novel candidates for the environmental control of photosynthesis

09:40 – 10:00 Robyn Phillips, University of Cambridge
The role of plastid to nucleus signalling in the evolution of C_4 photosynthesis

10:00 – 10:20 Gustaf Degen, Lancaster University
Exploiting diversity in the regulation of carbon assimilation to improve wheat productivity

10:20 – 10:50 Refreshment break (posters)

Session 4 **Chair: Cristina Sales, Lancaster University**

10:50 – 11:10 Ross-William Hendron, University of Oxford
Light spectra inform differential chloroplast development in C_4 photosynthetic leaves

11:10 – 11:30 Conor Simpson, University of Cambridge
Using natural variation within a species to understand C_4 photosynthesis

11:30 – 11:50 Nicky Atkinson, University of Edinburgh
Building a CCM in higher plants: An EPYC adventure

11:50 – 12:10 Indu Santhanagopalan, University of Cambridge
Diel regulation of EPYC1-Rubisco interactions in Chlamydomonas CCM

12:10 – 12:20 Group photo

12:20 – 13:20 Lunch (posters)

Plastid Preview 2018, Lancaster University, Sept 3rd – Sept 4th

Tuesday September 4th (cont.)

Session 5 **Chair: Gustaf Degen, Lancaster University**

13:20 – 13:40 Panupon Khumsupan, University of Edinburgh
Editing the nuclear-encoded Rubisco small subunits in Arabidopsis thaliana *with CRISPR/Cas9*

13:40 – 14:00 Ravendran Vasudevan, University of Edinburgh
CyanoGate: A Golden Gate modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax

14:00 – 14:20 Henry Taunt, University College London
Chlamy vs. *Malaria: The* Chlamydomonas reinhardtii *chloroplast as a production platform for insecticidal dsRNA*

14:20 – 14:40 Laura Wey, University of Cambridge
Synechocystis current output and physiological effect of harvesting extracellular electrons in a biophotovoltaic device

14:40 – 15:10 Refreshment break (posters)

Session 6 **Chair: Mareike Jezek, University of Glasgow**

15:10 – 15:30 Rona Costello, University of Oxford
Under new management: The dynamics of plastid proteome evolution

15:30 – 15:50 Sabri Mohd Ali, University of Oxford
Regulation of chloroplast protein import machinery by SP1-type ubiquitin E3 ligase

15:50 – 16:10 Robert Sowden, University of Oxford
Protein import in chloroplast-mediated immunity and stress tolerance

16:10 – 16:30 Poster/seminar prizes, concluding remarks

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High-throughput phenotypic exploration of photosynthetic traits; exploring novel genetic variation to promote high biomass and yield in wheat

Lorna McAusland[1], Hubbart-Edwards S[2], Atkinson J[1], King J[2], King I[2], Murchie E[1]

1. Division of Crop and Plant Science, University of Nottingham, Sutton Bonington, UK
2. Nottingham BBSRC Wheat Research Centre, Division of Crop and Plant Science, University of Nottingham, Sutton Bonington, UK

Wheat production has plateaued in many areas of the world due to a lack of novel genetic variation for agronomically important traits and is further compounded by the negative impacts of climate change. Distant relatives and landraces offer a valuable source of phenotypic traits lost in the evolution of modern varieties as a result of severe genetic bottlenecks. Recent advances in the ability to detect and characterise genetic material from crosses between distant relatives and elite lines (introgressions) provides powerful tools to rapidly introduce greater genome-wide variation. Rapid phenotyping of these introgressed lines is a vital step in rapidly identifying relevant traits that can be used for breeding and pre-breeding into elite varieties for improved biomass and yield. There is now recognition that improved grain yields of major crops require enhanced total dry weight production which must arise mostly from an improvement of radiation-use efficiency (RUE). Raising RUE, and therefore supporting higher biomass, requires a higher leaf and canopy photosynthesis rate and this remains an important target to underpin future yield progress. This presentation will discuss the progress in identifying photosynthetic variation between distant relatives of wheat and discuss a novel method developed at the University of Nottingham to screen for changes in photosynthetic efficiency using chlorophyll fluorescence in large populations of introgressed wheat lines.

Session 1, Speaker 2 Monday Sept 3rd 14:20 – 14:40

Potential photosynthesis is a key driver of yield in the Watkins wheat landrace collection

Cristina Sales[1], Molero G[2], Parry MAJ[1], Hall A[3], Carmo-Silva E[1]

1. Lancaster Environment Centre, Lancaster University, UK
2. International Maize and Wheat Improvement Center (CIMMYT), El Batán, Mexico
3. Earlham Institute, Norwich Research Park Innovation Centre, UK

Photosynthesis is the primary determinant of biomass production and is a target to increase the yield potential of wheat. In a panel of 64 UK cultivars grown in the field, flag leaf photosynthetic rates measured at pre-anthesis were greater in high-yielding than in low-yielding cultivars and correlated positively with both grain yield (GY) and with harvest index (HI). Whilst the genetic basis for photosynthetic efficiency remains largely unknown, many of the photosynthetic component traits were shown to be heritable and therefore amenable to phenotypic selection. Landraces are a potential source of trait diversity and the Watkins bread wheat collection has a high level of genetic diversity. In this study, we characterised under controlled conditions the photosynthetic properties of 97 members of the Watkins core collection, i.e., a refined selection of the original Watkins set that maintain the genetic diversity of the collection. Our results showed that flag leaf net CO_2 assimilation at booting stage under saturating light and CO_2 (A_{max}) correlated significantly and positively with GY and HI. A_{max} showed higher correlation than operational photosynthetic rate (ambient light and ambient CO_2 concentration), indicating that the A_{max} in the flag leaf is a better predictor of GY and HI in the Watkins wheat accessions grown in the glasshouse. Rubisco initial and total activity *in vitro* had a positive correlation with these yield components, and total activity showed higher correlation than initial. Combined, the results indicate that potential photosynthesis at flag leaf level is a key driver of grain yield in the Watkins wheat landrace collection highlighting the importance to increase photosynthesis to maximize yield potential. The phenotypic variation in photosynthetic traits observed in landraces can be exploited through the International Wheat Yield Partnership (http://iwyp.org/) to incorporate new sources of variation with greater photosynthetic potential into their breeding pipelines.

Session 1, Speaker 3 Monday Sept 3rd 14:40 – 15:00

Leaf gas-exchange measurement with spectral capture to detect temperature-induced spectral signatures: a new method tested on soy and tropical trees

Rakesh Tiwari[1], Foyer CH[2], Gloor E[1], Fauset S[1], Galbraith D[1]

1. School of Geography, University of Leeds, UK
2. Centre for Plant Sciences, University of Leeds, UK

The frequency and intensity of extreme temperatures in the tropics are increasing rapidly as a result of climate change. The responses of tropical trees to such "heat peaks" is poorly understood, particularly with regard to evergreen trees that retain leaves during these high-temperature conditions. To study these effects, we report measurements from a forest site at the southern boundary of the Amazon forests, where temperatures are amongst the highest and have increased particularly rapidly. Firstly, using tower-based canopy thermography during the dry season, we were able to identify a range of leaf temperature regulation strategies across canopy species. Some species maintained leaf temperatures higher than air whereas others closely tracked air temperature. Secondly, in recognition that temperature-dependent limitations on photosynthesis are largely understood in crop plants, but mechanisms that limit photosynthesis in tropical trees remain poorly characterized, we measured instantaneous leaf-level photosynthesis response curves on excised sun-exposed branches. The data reveal that some species could sustain CO_2 assimilation rates at temperatures as high as 35-40°C. While measuring CO_2 assimilation rate in the leaf chamber, we also captured leaf reflectance and absorptance spectra. This new method which we developed allows capture of leaf spectra on intact leaves. The method was first applied to soybean leaves where we found temperature induced signatures both in the visible and red - far-red wavelengths associated with PSII fluorescence emission. The potential of this method for assessing the physiological status of leaves *in vivo* will be discussed, together with the spectral signatures of Amazon trees.

Session 1, Speaker 4 Monday Sept 3rd 15:00 – 15:20

The fluctuating canopy light environment and implications for crop photosynthesis

Alexandra Burgess[1], Murchie E[1]

1. School of Biosciences, University of Nottingham, UK

Plant and crop canopies are complex, and the arrangement of plant material is critical in determining the spatial and temporal distribution of light. This is particularly true for lower canopy layers, where unpredictable light fluctuations (i.e. sun flecks) pose a conundrum to the photosynthetic machinery. Advances in computing power coupled with creation of novel techniques to capture plant architecture and model radiation has opened up new ways to explore the canopy light environment and link this to traits that limit productivity. Here we show results from two determinants of canopy productivity: photosynthetic acclimation (photoacclimation) and wind-induced movement. Photoacclimation is the process by which leaves alter their morphology and/ or biochemical capacity to cope with changes in light levels. An empirical model of photoacclimation was adapted to predict the optimal distribution of photosynthesis throughout architecturally contrasting wheat canopies. Discrepancies between the model predictions and measured data indicates that although the photosynthetic capacity of leaves is high enough to exploit brief periods of high light within the canopy (particularly towards the base), the frequency and duration of sun flecks are too small to make acclimation a viable strategy in terms of carbon gain. This suboptimal acclimation renders a large portion of residual photosynthetic capacity unused. Whilst the light environment within plant canopies has been partially characterised for static canopies, wind movement is very rarely accounted for. Evidence from both natural and agricultural systems has shown that minor canopy perturbations have the potential to significantly influence whole canopy photosynthesis by altering the light distribution reaching photosynthetic tissue. Movement will depend upon many different factors including architectural features, physiological status and the local environmental conditions.
Can targeting both crop biomechanical properties (i.e. movement in light winds) and biochemical capacity (i.e. response to a change in light levels) provide a means to improve canopy productivity?

Session 2, Speaker 1 Monday Sept 3rd 16:00 – 16:20

Simple strategies to promote you and your science

Mary Williams[1], Binder M[1], Cato S[1]

1. American Society of Plant Biologists, Rockville, MD, USA

When you think about all of the effort you put into your research, do you really want to leave it to chance that people find it and cite it, leading to the eventual offer of your dream job? If not, there are simple and agreeable ways that you can raise the visibility of you and your work, from developing a social network, engaging in outreach, or learning communication skills like basic graphic design, WordPress, and video creation. I'll also show you how the free and open Plantae community supports these efforts. Through Plantae, you can follow individuals, join networks, find resources, contribute to collections, share projects and identify collaborators. Early-career scientists can find opportunities to augment their CVs and demonstrate professional skills, for example by writing blog posts, posting videos, curating article collections and managing discussions. Plantae also hosts co-created projects and programs, including webinars (topics range from *Using WordPress* to *Computational Plant Science*), the Taproot Podcast series, the Self Reflection series, a weekly plant science research roundup, and the Conviron Scholars and Plantae Fellows programs. Hopefully I will inspire you to try something new, extend your skills, build recognition, and find success!

Session 2, Speaker 2 Monday Sept 3rd 16:20 – 16:40

Promoting plant science and growing connections

Mike Whitfield[1]

1. New Phytologist Trust, UK

You've written the perfect paper, and now you want to share it with the world. How can you help your research to stand proud from the undergrowth?
In this talk, I'll share some tips and tricks to help your paper to break through the canopy. From Altmetric to Zenodo, I'll highlight some of the tools you can use to ensure that your research is reaching the audiences it needs to.
Our mission at the New Phytologist Trust is to promote plant science; I'll introduce you to some of the ways in which we're working to broaden the reach of plant science research published in our two journals, and invite you to ask what your journal can do for you.

Session 3, Speaker 1 Tuesday Sept 4th 09:00 – 09:20

Plastid fate at the initial stage of isolated microspore culture in spring barley

Monika Gajecka[1], Marzec M[1], Chmielewska B[1], Jelonek J[1], Zbieszczyk J[1], Szarejko I[1]

1. University of Silesia in Katowice, Poland

It is possible to change the pathway of microspore development from the gametophic to the sporophytic program through *in vitro* culture of anthers or isolated microspores. This process, called androgenesis, leads to the production of double haploid plants, which have a great practical potential in plant breeding programs, and allows to speed up the production of new varieties. However, androgenesis is a highly genotype-depend process whose effectiveness in cereals is strongly reduced by the occurrence of albino plantlets among regenerants. It was shown that albino regenerants contain plastids arrested at early stage of development, but despite many ultrastructural and molecular studies, the basic mechanism of albino plantlet formation during androgenesis has not been identified. Furthermore, the genotype-dependence of albino regeneration remains to be explained.

In the presented study, the plastid development and differentiation during pollen maturation and the fate of differentiated plastids after pretreatment were studied in two spring barley cultivars that differed extremely in the rate of green to albino plantlet regeneration. Additionally, the expression level of genes related to plastid biogenesis, differentiation and degradation was examined during the first week of microspore culture. Both cultivars differed in terms of expression profiles of analysed genes and plastid development during pollen maturation *in vivo* and the initial stages of *in vitro* culture. Cultivar 'Mercada' that regenerated mostly albino plantlets showed much faster differentiation of proplastids into amyloplast during pollen maturation than cultivar 'Jersey' that produced mostly green regenerants. Additionally, the expression analysis revealed the activation of genes related to plastid dedifferentiation in cultivar 'Jersey' which was not observed in cultivar 'Mercada'. It can be concluded that the genotype ability to regenerate green plantlets is related to amyloplast differentiation/dedifferentiation during microspore development *in vivo* and the first stages in culture *in vitro*.

Chloroplast RNA-binding proteins (cpRNP), novel candidates for the environmental control of photosynthesis

Jonathan Griffin[1], Prado K[2], Halliday KJ[2], Toledo-Ortiz G[1]

1. Lancaster University, Lancaster Environment Centre, Bailrigg Lancaster, UK
2. University of Edinburgh, School of Biological Sciences, King's Buildings, Max Born Crescent, Rutherford Building, UK

Photosynthesis is fundamental to modern life and society through biomass production. This process is regulated by light- and thermo-sensitive phytochrome photoreceptors (Phy). Phy synchronizes plant development to a changing environment by orchestrating large-scale gene expression changes. This study aims to explore a novel function of Phy in controlling chloroplast RNA processing. A potential pathway was identified involving the Chloroplast RNA-binding proteins (cpRNPs). This family performs post-transcriptional processing of chloroplast RNAs and are sensitive to environmental stress conditions. We have established that cpRNPs gene expression is light sensitive and Phy-dependent. cpRNPs are also conserved in many plant species, hinting at an essential role. To evaluate the potential role of cpRNP in integration of light and temperature signals, selected *Arabidopsis thaliana* mutants were examined in different physiological temperatures to elucidate their potential roles. During deetiolation, cpRNP mutants show clear alterations in greening and in the accumulation of chloroplast genome derived-photosynthesis associated mRNAs. The role of these proteins is not exclusive of light to dark transition stages: cpRNP mutant seedlings also showed reductions in biomass and chlorophyll accumulation in warm and cold conditions. These phenotypes point at cpRNP roles in temperature acclimation of greening responses and maintenance of chloroplast metabolism. To investigate the role of light and temperature on the accumulation and distribution of cpRNPs in the chloroplast we used a cell biological approach based on GFP-tagged cpRNPs and confocal microscopy. By characterizing the cpRNPs we aim to elucidate novel roles of Phy in coordinating chloroplast functions, photosynthesis, and environmental stimuli acclimation mechanisms.

The role of plastid to nucleus signalling in the evolution of C_4 photosynthesis

Robyn Phillips[1], Hibberd JM[1]

1. Department of Plant Sciences, University of Cambridge, UK

C_4 photosynthesis has evolved from the ancestral C_3 state in over sixty lineages of plants. Although commonly described as the most remarkable example of convergent evolution known to biology, more recent analysis indicates that the C_4 pathway is also underpinned by parallel evolution. For example, orthologous genes from separate C_4 lineages have repeatedly been recruited into the C_4 pathway such that they are co-regulated with existing genes of C_3 photosynthesis. For decades, the mechanisms allowing co-regulation of C_4 and C_3 photosynthesis genes have been unclear, but it was recently shown that in C_3 *Arabidopsis thaliana* seven of sixteen C_4 orthologues are already controlled by plastid-to-nucleus signalling. This strongly implies that evolution has re-enforced existing regulatory networks that operate in the C_3 state to control expression of C_4 genes. Currently, the extent to which plastid-to-nucleus signalling regulates C_4 orthologues in the C_3 state is unknown. To investigate this, *Arabidopsis thaliana* and *Oryza sativa* were subjected to inhibitors of chloroplast development and differential gene expression analysis performed. First, the concentrations of both lincomycin and norflurazon leading to repression of chloroplast development were established. Comparisons between illuminated and dark-grown plants for each treatment enabled the complementary roles of plastid-to-nucleus signalling and light regulation to be identified. Over 20,000 genes were detected for each sample, with over 1000 genes differentially expressed in lincomycin treated plants compared to controls, and over 3500 genes differentially expressed when plants were treated with norflurazon (p-adj < 0.05). Gene Ontology terms including photosynthesis and a variety of cellular metabolic processes were over-represented in these datasets. Furthermore, over half of the C_4 orthologues were regulated by both light and the chloroplast in both species. Future work will aim to elucidate the regulatory mechanisms involved, and to gain a greater understanding of the temporal variation involved in retrograde signalling.

Exploiting diversity in the regulation of carbon assimilation to improve wheat productivity

Gustaf Degen[1], Carmo-Silva E[1]

1. Lancaster Environment Centre, Lancaster University, UK

Rubisco is the central enzyme in the carbon reactions of photosynthesis. However, it is prone to inhibition by naturally occurring sugar phosphates, amongst others its own substrate RuBP when it binds tightly to the inactive uncarbamylated active site of Rubisco. The levels and potency of inhibitory sugar phosphates vary between time of the day and are dependent upon environmental factors, such as temperature.

In order to remove these, and to reactivate Rubisco, the molecular chaperone Rubisco activase (Rca) is required, which uses the energy from ATP hydrolysis to remodel the active site of Rubisco to release the inhibitors. In wheat, two genes, Rca1 and Rca2, are present. Rca1 produces the short Rca1β isoform and Rca2 yields the short Rca2β and the long Rca2α *via* alternative splicing.

It is well documented that Rca is a thermosensitive protein and ceases to function at moderately high temperatures in species like Arabidopsis or tobacco, resulting in a decrease in photosynthesis. However, species adapted to warmer regions show increased thermal stability of activase. Due to projected increases in temperature as a result of climate change, wheat yields are predicted to decrease.

ATPase temperature assays conducted thus far showed different activities of the three isoforms from the B genome. Furthermore, the effect of heat stress on activase protein abundance and composition will give insight into the limitations activase poses on photosynthesis at moderately high temperatures.

The aim is to gain a better understanding of the effect of temperature on Rubisco activation in wheat and to identify superior activase isoforms in wild relatives to make wheat more resilient to climate change.

Light spectra inform differential chloroplast development in C_4 photosynthetic leaves

Ross-William Hendron[1], Kelly S[1]

1. Department of Plant Sciences, Oxford University, UK

Plants coordinate the expression of genes required to conduct photosynthesis in response to growth, metabolism, and changes in the environment. In species that have evolved two-cell C_4 photosynthesis, the expression of photosynthesis genes is partitioned such that leaf mesophyll and vascular sheath cells accumulate different components of the photosynthetic pathway. Although the biochemical differences that distinguish these two cell types are well defined, the identity of the regulatory networks and developmental mechanisms that facilitate this partitioning are unknown. Here evidence is provided that light spectrum differences between mesophyll and vascular sheath cells plays a role in facilitating the differential regulation and accumulation of photosynthesis genes in the C_4 plants. Across two independent origins of C_4 photosynthesis we show that the outer mesophyll cell layer preferentially accumulates transcripts encoding regulatory components of blue light signalling networks while the inner bundle sheath layer preferentially accumulates transcripts encoding red light signalling components. This regulatory network partitioning is consistent with the biophysical filtering of light as it passes through successive cell layers within the leaf. Through spectrum-specific de-etiolation experiments in dark grown seedlings, it is shown that transcriptional regulation of the mesophyll cell photosynthesis apparatus is differentially sensitive to blue and red light stimuli in C_4 maize leaves, but not in C_3 barley leaves. Together these findings provide evidence that light spectrum differences inherent in Kranz-anatomy provide a biophysical signal that has been exploited to facilitate photosynthetic partitioning in C_4 species.

Using natural variation within a species to understand C_4 photosynthesis

Conor Simpson[1], Singh P[1], Reeves G[1], Hibberd JM[1]

1. Department of Plant Sciences, University of Cambridge, UK

C_4 photosynthesis is a highly complex trait that requires changes in leaf biochemistry, morphology and cell biology. This includes alterations to the plastid complement of mesophyll and bundle sheath cells in leaves. Until recently, there were no reports of significant natural variation within a species and so limited insight into the C_4 pathway has been gained from classical genetics. Through analysis of various accessions of the C_4 model plant *Gynandropsis gynandra*, we found significant intraspecific variation for C_4 traits spanning gene expression to leaf morphology. These accessions are sexually compatible and therefore this variation can be exploited to measure heritability and initiate trait mapping for C_4 photosynthesis. I will present an outline of my project that involves using quantitative genetics and appropriate breeding models to calculate the hereditability of key traits associated with C_4 photosynthesis, and then approaches that will be used to undertake trait-mapping.

Building a CCM in higher plants: an EPYC adventure

Nicky Atkinson[1], Velanis C[1], McCormick AJ[1]

1. Institute for Molecular Plant Sciences, University of Edinburgh, UK

Photosynthetic efficiency in C_3 plants is restricted by the low specificity of Rubisco for CO_2 over O_2. Addition of a carbon concentrating mechanism (CCM) could greatly enhance photosynthetic output and therefore productivity, and an attractive model is that of the green algae *Chlamydomonas reinhardtii*. Building an algal-based CCM in higher plants will require the introduction of a suite of bicarbonate transporters, carbonic anhydrases, Rubisco aggregation factors and accessory proteins. We are currently characterising several known and newly discovered components, individually and in combination, with the aim of establishing the minimal set required for a functional and transferable CCM. A crucial component of the Chlamydomonas CCM is EPYC1, which is thought to aggregate Rubisco in the pyrenoid. We characterised the interaction between EPYC1 and the Rubisco small subunit (SSU) in yeast and *in planta*. EPYC1 interacted strongly with the Chlamydomonas SSU RbcS2 in a yeast two-hybrid system, but not with the Arabidopsis SSU RbcS1A. However, interaction was facilitated by the modification of RbcS1A to include alpha-helices from the RbcS2. A synthetic EPYC1 comprising duplications of key interaction domains had an even stronger affinity for SSUs. Surprisingly, EPYC1 required a plant-native chloroplast transit peptide to localise to the chloroplast in *Nicotiana benthamiana* and Arabidopsis, but expression was not detrimental to plant growth. In addition, Rubisco co-immunoprecipitated with EPYC1 in Arabidopsis SSU mutants expressing EPYC1 and RbcS2. We also aim to establish a functional bicarbonate transport system in higher plants, by co-expressing the transporter HLA3 with known interactors, and characterising a newly-discovered family of bestrophin-like proteins that are putatively involved in bicarbonate flux in the chloroplast. Early results show the latter localise to the chloroplast in a higher plant, thus creating an exciting platform for investigating their interactions with chloroplastic CCM components such as EPYC1.

Diel regulation of EPYC1-Rubisco interactions in Chlamydomonas CCM

Indu Santhanagopalan[1], Yadav A[2], Yadav G[2], Meyer M[3], Zhang Y[1], Griffiths H[1]

1. University of Cambridge, Cambridge, UK
2. National Institute of Plant Genome Research, New Delhi, India
3. Princeton University, Princeton, NJ, USA

Carbon concentration mechanisms (CCM) are employed by several aquatic phytoplanktons to increase photosynthetic efficiency in an environment limiting of CO_2 levels. *Chlamydomonas reinhardtii* is a unicellular alga that uses biophysical CCM in conjugation with Calvin Benson cycle and electron transport chain that are almost identical to those in higher plants. Chlamydomonas like several algae carry a proteinaceous organelle rich in Rubisco molecules called pyrenoid in their chloroplast. Enclosed by starch sheath and enmeshed in a tubule network continuous with thylakoids, pyrenoids are highly dynamic undergoing changes in composition, compaction and size during the day-night cycle. The changes in Rubisco aggregation within pyrenoids is hypothesized to be regulated by its interaction with a protein called EPYC1. Experimental evidence showing EPYC1-Rubisco interactions with in the pyrenoids led to the proposal for Rubisco-linker like role for EPYC1. EPYC1 is a 35 kDa serine-rich, highly basic protein made of four nearly identical ~60 residue repeats, which undergoes extensive phosphorylation. Diurnal changes in CCM and pyrenoids, and CO_2-level dependent EPYC1 phosphorylation state (Yi Zhang and Mortiz Meyer-personal communication) led us to hypothesize that the EPYC1 phosphorylation state is a regulator of interaction with Rubisco. We are using synchronized Chlamydomonas cells to examine the diel changes in EPYC1 phosphorylation by electrophoretic and mass spectrometric studies. We have used diurnal transcriptomic data for Chlamydomonas to identify potential kinases and phosphatases that regulate EPYC1 phosphorylation based on their expression profiles. We are currently characterizing knockout strains for these potential EPYC1 phosphorylation regulators to examine their role in CCM and pyrenoid dynamics. Our studies are providing new insights regarding diurnal regulation of algal CCM, identifying new CCM components, and helping us move closer to establishing CCM in higher plants.

Editing the nuclear-encoded Rubisco small subunits in *Arabidopsis thaliana* with CRISPR/Cas9

Panupon Khumsupan[1], McCormick AJ[1]

1. SynthSys & Institute of Molecular Plant Sciences, University of Edinburgh, UK

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is responsible for net photosynthetic carbon fixation in plants. The enzyme consists of a chloroplast-encoded large subunit and typically a family of nuclear-encoded small subunits. The roles of small subunit families are still somewhat unclear but different isoforms may play specific roles during development or regulation of Rubisco catalysis. To characterise the small subunit family in the model plant *Arabidopsis thaliana* (Arabidopsis), small subunit knock-out mutants were generated using CRISPR/Cas9. Arabidopsis contains four small subunit genes, *RbcS1A* located on chromosome 1, and *RbcS1B*, *RbcS2B* and *RbcS3B* located in tandem on chromosome 5. Two pairs of sgRNAs were designed to target each small subunit gene. The deletion efficiency of sgRNA pairs was examined in mesophyll protoplasts. Using Cas9 fused to YFP, Cas9 was expressed 24 h post-transfection and deletion sizes between 96-180 bp were detected. The T1 plants were screened by PCR and sequenced to detect deletions induced by sgRNAs (deletion band) and showed a deletion efficiency ranging from 0-14%. We found that point mutations occurred at a rate of 0-31%. Sequencing of transgene-free T2 plants revealed that point mutations had been inherited. Current efforts are focused on generating double and triple knock-out mutants and developing Arabidopsis as a platform for examining the contribution of the small subunit to Rubisco catalysis.

Session 5, Speaker 2 Tuesday Sept 4th 13:40 – 14:00

CyanoGate: A Golden Gate modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax

Ravendran Vasudevan[1,2], Gale G[1,2], Schiavon AO[1,2], Wang B[2], Howe CJ[3], McCormick AJ[1,2]

1. Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, Edinburgh, UK
2. SynthSys, School of Biological Sciences, University of Edinburgh, Edinburgh, UK
3. Department of Biochemistry, University of Cambridge, Hopkins Building, Downing Site, UK

Cyanobacteria are the photosynthetic bacteria capable of converting sun energy in to chemical energy. Considerable investment has been deployed to bring cyanobacterial culturing technologies to commercial scale for the production of high values products. Transformation tools have been developed for generating marked and unmarked mutant in several cyanobacterial strains. More recently, marker-less strains have been generated using CRISPR-based approaches. Although exciting progress is being made in developing effective transformation systems, cyanobacteria are still lagging behind in the field of synthetic biology compared to bacterial and mammalian systems. Only a few broad-host range genetic parts have been characterised, while part libraries for constructing regulatory modules and circuits are only starting to become available, or are still being developed. Here we have developed a system to unite cyanobacteria with plant systems, by building on an established Golden Gate MoClo syntax and assembly library, which we have called the CyanoGate system. Firstly, we have constructed and characterised a suite of new level 0 parts for use in cyanobacteria, several of which are promoters (42 promoters) and terminators (22 terminators). Secondly, we have constructed an additional level (Level T) acceptor plasmids for chromosomal integration (suicide vector) and or self-replication. Finally, we have constructed 7 neutral site vectors for chromosomal over expression studies, and we have characterised 1 new origin of replication that replicates in cyanobacteria to increase the dynamic range available to researchers.

Chlamy *vs.* Malaria: the *Chlamydomonas reinhardtii* chloroplast as a production platform for insecticidal dsRNA

Henry Taunt[1], D'Archimbaud A[1], Purton S[1]

1. University College London, Gower Street, London, UK

The *C. reinhardtii* chloroplast has long been used as a production platform for recombinant proteins; however, to date very little attention has been paid to double stranded RNA synthesis and accumulation in the algal chloroplast. In eukaryotic organisms dsRNA is interpreted as an attack by RNA viral particles, and as such is processed into micro RNAs which then efficiently inhibit the target sequence. This is the basis of intracellular RNAi knockdown technologies, and such RNAs have also been shown to have extracellular activities against numerous organisms.
The *C. reinhardtii* chloroplast offers a unique platform for production of dsRNAs as they can be efficiently transcribed by the chloroplast transcription machinery, but in the absence of any processing mechanism are able to accumulate in an intact state. Previous works have demonstrated production of dsRNA in the *C. reinhardtii* chloroplast using stem loop hairpin structures, but these require large inverted repeats which can be difficult to synthesise or construct by methods such as PCR.
Here we present a novel system for the production of dsRNA in the *C. reinhardtii* chloroplast whereby an unmodified target sequence can be inserted directly into the vector by a single step Golden Gate reaction. Demonstration of the technology with a dsRNA previously shown to inhibit the malaria carrying mosquito *Anopheles gambiae* will also be presented.

Synechocystis current output and physiological effect of harvesting extracellular electrons in a biophotovoltaic device

Laura Wey[1], Rabideau C[1], Bombelli P[1], Howe CJ[1]

1. Department of Biochemistry, University of Cambridge, UK

Certain cyanobacteria including the model species *Synechocystis* sp. PCC 6803 exhibit the ability to export electrons outside the cell, called exoelectrogenic activity. There is great potential to harness cyanobacterial exoelectrogenic activity in biophotovoltaic devices to renewably generate electricity, but power outputs remain low. Therefore, an overarching aim of this research is to identify a highly exoelectrogenic cyanobacterial strain for application in biophotovoltaic devices. Further, the transport pathways of electrons initially derived from photosynthesis between the thylakoid and cytoplasmic membranes and outside the cell remain poorly defined. Thus, this research also aims to increase understanding of the biological basis of cyanobacterial exoelectrogenic activity. The three candidates I am focusing on in this project are electron sinks, type IV pili and the surface layer (S-layer). Respiratory terminal oxidases and flavodiiron proteins are protective electron sinks that transfer excess electrons to O_2, competing for electrons with the anode in a biophotovoltaic device. There is evidence that type IV pili are electrically conductive microbial nanowires. The S-layer is the most peripheral layer of the cell, hypothesised to be electrically insulating. Synechocystis mutants lacking candidates and wild type were held at a bias potential in a biophotovoltaic device and the current output profile over time under different light and dark cycling regimes was recorded. The effects of the mutations and harvesting extracellular electrons on cyanobacterial cell sedimentation, biofilm formation and physiology were measured. This presentation communicates results of preliminary studies to identify a cyanobacterial mutant with heightened exoelectrogenic activity and the optimal growth conditions to achieve increased peak power output of biophotovoltaic devices.

Session 6, Speaker 1 Tuesday Sept 4th 15:10 – 15:30

Under new management: the dynamics of plastid proteome evolution

Rona Costello[1], Kelly S[1]

1. Department of Plant Sciences, University of Oxford, UK

Diversity in plastid form and function in the plant kingdom is in part a product of differences in the protein content of plastids among plant species. Changes to the plastid proteome during evolution can occur through alterations in the plastid genome, or *via* the gain (or loss) of plastid targeting of nuclear-encoded proteins. However, it is unknown how prevalent such gains and losses have been during plant evolution, or the contribution that they've had on the divergence of different plant lineages. Here we chart the evolution of the plastid proteome in land plants by mapping the gain and loss of plastid targeting among nuclear-encoded proteins. This study revealed that there have been substantial changes in the composition of chloroplast proteomes throughout plant evolution. It is shown that changes in protein targeting were most common in genes with regulatory functions suggesting that diversification in the chloroplast regulatory network has been the most prevalent form of chloroplast evolution. Furthermore, we show that gain or loss in plastid targeting is more likely to happen following gene duplication. In summary, this work provides new insight into mechanism and dynamics of plastid evolution during the radiation of land plants.

Session 6, Speaker 2 Tuesday Sept 4th 15:30 – 15:50

Regulation of chloroplast protein import machinery by SP1-type ubiquitin E3 ligase

Sabri Mohd. Ali[1], Ling Q[1], Jarvis P[1]

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Chloroplast are dynamic organelle which rely on the import of thousands of nuclear-encoded proteins for their function. The process of import is mediated by the Translocon complex located on the Outer and Inner membrane of the Chloroplast (TOC/TIC). In 2012, we identified a gene called *SP1* (*suppressor of ppi1 locus1*) through a forward genetic screen of an Arabidopsis protein import mutant, *ppi1* (*plastid protein import1*). The *SP1* gene encodes a key RING E3 ligase enzyme in the Ubiquitin Proteosome-System (UPS) protein degradation pathway, important during the organelle transition process as well as abiotic stress responses. There are two homologues of *SP1* in Arabidopsis, *SPL1* and *SPL2*. Previously we showed the overexpression of *SPL1* was not able to complement the *sp1* mutation in the *ppi1* background. This suggests that SPL1 may have a distinct role to SP1 and although less is known about SPL2. A phylogenetic analysis of protein sequences indicate early divergence of SP1 and SPL2 into their monophyletic group from a common ancestor. SPL1 arose from a recent duplication event within the SP1 group and biochemical analysis shows loss of ubiquitination activity in SPL1. The result from genetic analysis suggests SPL2 to be partially redundant to SP1. These findings give insight to a complex network of chloroplast protein import machinery regulation by E3 ligases which will pave a way for better engineering stress tolerant crops.

Session 6, Speaker 3 Tuesday Sept 4th 15:50 – 16:10

Protein import in chloroplast-mediated immunity and stress tolerance

Robert Sowden[1], Preston GM[1], Jarvis P[1]

1. Department of Plant Sciences, University of Oxford, UK

Chloroplasts are the defining feature of plant cells. Not just photosynthesis machines, they are signalling hubs engaged in a continuous conversation with the nucleus and other organelles. In environmental stress, chloroplasts must be carefully regulated to protect plants from harmful photosynthetic by-products. Recently, there has been a growing body of work recognising the vital roles the chloroplast has to play in plant immunity. In disease, pathogens compete with the plant to alter chloroplast function in order to engender susceptibility.

Chloroplast function depends on its proteome, much of which arises from nucleus-encoded genes. Nucleus-encoded chloroplast proteins are then delivered to the chloroplast through a small number of import pathways. Likewise, some pathogen effectors are imported to the chloroplast or induce changes in host protein localisation.

I am exploring the regulation of chloroplast protein import as a key step in successful pathogenesis, causing chloroplast-mediated immunity to become derailed and enhancing susceptibility to disease. The chloroplast is a point of convergence both for competing immunity and stress tolerance pathways, and for the competing interests of the plant and the pathogen – and this may be an important consideration in the quest for designer climate change-resistant crops.

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2. Jessica Bampton, University of Southampton
   Singlet oxygen signalling pathways during de-etiolation in Arabidopsis
3. Ciaran Griffin, University of Plymouth
   Characterisation of chloroplast behaviour during rice blast disease progression
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5. Pracha Tree-intong, University of Nottingham
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11. Alexandros Vardakis, University of Edinburgh
    Feeding Rubisco with CO_2: modifying chloroplastic carbonic anhydrase activity in Arabidopsis thaliana *for introducing an algal CO_2-concentrating mechanism*
12. Rachel Taylor, University of Leeds
    Characterising WHIRLY1 functions in plastids
13. Yousef Al-Hajaya, University of Leeds
    The role of catalase enzyme subcellular localization in plant development and stress responses
14. Nurhayati Razak, University of Leeds
    Chloroplast development in barley leaves is controlled by WHIRLY1
15. Mareike Jezek, University of Glasgow
    Bicarbonate enrichment of chloroplasts via *the mammalian anion exchanger AE1*
16. Katie Schulz, University of Leeds
    Determining the molecular and metabolic mechanisms that underpin the blackening phenomenon in cut carrots
17. Chun Sing Lau, University of York
    The search for the elusive thylakoid membrane bicarbonate transporter of Chlamydomonas reinhardtii
18. Emmanuel Gonzalez-Escobar, Lancaster University
    Investigating the regulation of NADP-malic enzyme in C_4 monocot grasses during dark–light transitions
19. Mary Williams, ASPB
    Plantae.org: a place to connect, curate, collaborate, and co-create

POSTER 1

Characterisation of thylakoid membrane bicarbonate transporters in the *Chlamydomonas reinhardtii* CO_2 concentrating mechanism

Charlotte Walker[1], Lau CS[1], Yates G[1], Emrich-Mills T[1], Grouneva I[1], Mackinder LCM[1]

1. University of York, Department of Biology, York, UK

Eukaryotic algae are responsible for approximately one-third of global carbon fixation. Many algae enhance their photosynthetic output by operating a CO_2 concentrating mechanism (CCM) under low CO_2 conditions to enhance the carboxylation reaction of Rubisco. The green alga *Chlamydomonas reinhardtii* has been established as a model organism for elucidating the mechanisms behind the algal CCM. Previous research on the Chlamydomonas CCM has established Carbonic Anhydrase 3 (CAH3) as an essential component located within the pyrenoid, a cellular microcompartment containing tightly packed Rubisco and permeated by thylakoid tubules. CAH3 is an α-carbonic anhydrase, converting HCO_3^- to CO_2 in the thylakoid lumen during CCM activation. As a result, there is a need for a thylakoid membrane HCO_3^- transport protein to shuttle HCO_3^- from the chloroplast stroma into the thylakoid lumen for a functional CCM. A recent study by Mackinder *et al.* (2017) developed a high-throughput fluorescence protein tagging and affinity purification pipeline for Chlamydomonas. This approach yielded potential candidates for HCO_3^- transport across thylakoid membranes, including a number of bestrophin-like proteins. In this study we undertake the characterisation of these HCO_3^- transport candidate proteins in order to fully understand this crucial step in the Chlamydomonas CCM.

POSTER 2

Singlet oxygen signalling pathways during de-etiolation in Arabidopsis

Jessica Bampton[1], Page MT[1], Garcia-Becerra T[1], McCormac AC[1], Stephenson PG[1], Ling Q[3], Takagi H[4], Okamoto H[1], Smith AG[2], Jarvis P[3], Terauchi R[4], Terry MJ[1]

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4. Iwate Biotechnology Research Center, Kitakami, Japan

During de-etiolation there is a requirement for rapid synthesis of chlorophyll and the photosynthetic apparatus in the developing chloroplast. Since most chloroplast proteins are encoded in the nucleus, retrograde signals from the chloroplast are needed to enable this process to proceed safely and efficiently. One molecule implicated as a retrograde signal is singlet oxygen ($^1O_2$), which is produced by chlorophyll and its biosynthetic pathway intermediates when excited by light. We have previously shown using *Arabidopsis thaliana* that $^1O_2$ production from one of these intermediates, protochlorophyllide (Pchlide), downregulates genes encoding chlorophyll biosynthesis enzymes and components of the photosynthetic apparatus. Here we describe an EMS mutagenesis screen to identify potential components of this $^1O_2$ signalling pathway. Seedlings grown in far-red light over several days accumulate Pchlide, and upon exposure to white light produce a $^1O_2$ burst, which causes downregulation of the chlorophyll biosynthesis gene HEMA1. Transgenic seedlings were produced that contain the HEMA1 promoter linked to the BAR gene, which confers phosphinothricin (PPT) resistance. Mutants that retained HEMA1 (and therefore BAR) expression following a far-red screen on PPT-containing media survived and were termed *safe after far-red* (*saf*). One of the most promising mutants, *saf7*, maintained WT levels of Pchlide, greened, and was able to maintain expression of chlorophyll biosynthesis and photosynthesis genes following $^1O_2$ production. The *saf7* mutant was identified as having a mutation in the *toc132* gene by next generation sequencing. The *toc132* mutant showed a *saf* phenotype following a far-red screen and western blotting analysis confirmed that *saf7* had a reduced level of truncated TOC132. These results suggest that TOC132 may play a role in $^1O_2$ signalling during de-etiolation and we will report on progress made in elucidating this role.

POSTER 3

Characterisation of chloroplast behaviour during rice blast disease progression

Ciaran Griffin[1], Castillo-Vila S[2], Lopez-Llorca LV[2], Yan X[3,4], Talbot NJ[3,4], Littlejohn GR[1]

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2. University of Alicante, Spain
3. School of Biosciences, University of Exeter, UK
4. Current address, Sainsbury Laboratory, Norwich, UK

Development of strategies to control plant pathogens are crucially dependent on a firm understanding of the interaction between both partners of the pathosystem. Recent studies suggest that the chloroplast may be a common target for a range of different pathogens and their effectors, which is entirely expected, given the importance of the chloroplast in primary metabolism and its emerging role in plant immunity. Preliminary data shows that chlorophyll fluorescence parameters in rice leaves are altered early in rice infection by the rice blast-causing ascomycete, *Magnaporthe oryzae*. Further observations suggest that chloroplasts in infected tissue are less mobile than in uninfected cells and associate with invasive hyphae. This suggests the intriguing possibility that in addition to delivering effector proteins to the plant cell, which may directly influence chloroplast movement and function, hyphae may physically interact with host organelles. The aims of this project, therefore, are to test the basis of interaction between the partners in the rice blast model pathosystem by determining potential degradation in chloroplast structure and function during infection and to carrying out imaging of hyphal growth and interaction with chloroplasts using TEM in order to establish the timing and nature of chloroplast-hyphal interaction.

POSTER 4

Using protective non-photochemical quenching (pNPQ) for assessing rice canopy productivity

CC Foo[1], Burgess A[1], Ruban A[2] and Murchie E[1]

1. Division of Plant and Crop Science, School of Biosciences, University of Nottingham, UK
2. Department of Cell and Molecular Biology, School of Biological and Chemical Sciences, Queen Mary University of London, UK

Plants have a love/hate relationship with light. Plants require light to convert carbon dioxide into food (photosynthesis). However, at increasing light levels, net photosynthesis is reduced and reaches a plateau, resulting in excessive excitation energy in pigment protein complexes. The excess energy would harm the reaction centres resulting in a condition called photoinhibition. Plants remove this excess energy *via* a harmless pathway called non-photochemical quenching (NPQ). However, it is not known how much of NPQ is actually protective and this is important since it momentarily reduces the quantum yield of photosynthesis resulting in a potential loss in productivity. Studying pNPQ (protective NPQ) at the canopy level is important for crop canopy productivity. So far, pNPQ has only been used for work with model plants such as *Arabidopsis thaliana*.

pNPQ can now be quantified in a non-destructive manner using dark-adapted photochemical quenching (qPd). qP denotes the number of photosystem II (PSII) reaction centres that are intact and open. In the absence of photoinhibition, qPd (measured in the dark) is 1. The onset of photoinhibition is denoted by a qPd value less than 1. This allows early detection of onset of photoinhibition during different ontogenetic phases in all plants *in vivo*. Hence, when translated to canopies, it could be used to accurately estimate light tolerance level simultaneously in leaves at different states of light saturation.

Rice canopies with different expression levels of the photoprotective protein PsbS were used. Youngest fully emerged leaves were dark adapted for 45 minutes prior to fluorescence measurements under different actinic light settings using a Walz Junior PAM. Calculations for ϕPSII and qPd were made, and we show that the qPd method works effectively in rice.

POSTER 5

Searching for useful variation in abiotic stress tolerance and photoprotection in accession of *Oryza glaberrima*

Pracha Tree-intong[1], Mayes S[1], Swarup R[1], Murchie E[1]

1. Plant and Crop Science Division, School of Bioscience, University of Nottingham, Sutton Bonington Campus, UK

Excessive light causes photoinhibition that can limit plant photosynthetic activity, growth and productivity. To minimise net photoinhibition, plants have established various photoprotection mechanisms to process light energy safely. It can occur *via* the metabolic processing of absorbed light energy to avoid oxidative stress or by leaf and chloroplast movement to optimize the interception and absorption of light. The aim of this investigation is to search for useful variations in abiotic stress tolerance linked with 155 accessions of African rice (*O. glaberrima*) which is a new genetic resource for improved tolerance to biotic and abiotic stress in comparison to Asian rice. An induction assay was performed raising the PPFD following a dark adaptation to 1500 μmol.m.$^{-2}$s.$^{-1}$ for 15 minutes, then returning to 100 μmol.m.$^{-2}$s.$^{-1}$ for 5 minutes using a portable LI-COR LI-6400 to identify those accessions having high photosynthesis, optimal photoprotective mechanisms and optimal kinetics of both. A preliminary screening showed substantial variations in both steady state values and the dynamics of change. High photosynthesis (Amax) was associated with high stomatal conductance and ETR among the 155 accessions. The correlations between Amax, gs, non-photochemical quenching (NPQ) and ETR lead to the understanding of the photosynthetic performance and some trade-offs e.g. transpiration (water loss) which is a vital trait as Leaf Water Use Efficiency. High NPQ could dissipate the excess incoming photon energy and prevent damage to the photochemical pathway but slow kinetics may result in a reduction of integrated photosynthesis. Twenty-three lines were selected for further study and it will be useful for the next process to understand the cause of the variation in photosynthetic efficiency.

POSTER 6

Recombination-mediated approach to cloning large, complex genes in the green alga *Chlamydomonas reinhardtii*

Tom Emrich-Mills[1], Mackinder L[1]

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Chlamydomonas reinhardtii is a fresh water alga used as a model organism for studying photosynthesis, genome evolution, and flagella function in eukaryotes. The species has a strong genetic platform, is relatively easy to culture in the lab, and has a recently available genome-wide mutant library and extensive online resources to aid researchers. However, the cloning of Chlamydomonas genes for mutant complementation and the generation of fluorescence protein fusion constructs for localisation has been problematic due to the high GC content and repetitive nature of the nuclear genome. Here we show efficient cloning of large, complex Chlamydomonas genes to be possible through a recombination-mediated approach. The technique utilises homologous recombination in *E. coli* to facilitate the cloning of genes from Bacterial Artificial Chromosomes containing fragments of the Chlamydomonas genome. Initial results indicate expression of cloned genes, tagged with a GFP derivative, can be achieved with their native promoters intact. Progress is being made to clone the putative components of the Chlamydomonas CO_2 concentrating mechanism (CCM), enabling their localisation and functional investigation. The algal CCM is based on active assimilation and concentration of CO_2 around the CO_2-fixing enzyme RuBisCO. A deeper understanding of this process may enable the engineering of a CCM into higher plants to enhance photosynthesis and increase yields.

POSTER 7

Improving wheat photosynthesis through wide crossing using wild relatives and selection using sub-ambient CO_2

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Wheat yield gains are currently insufficient to meet the demands of the future thus posing a risk to global food security. In order to increase yields it is necessary to improve photosynthesis, as this is the primary determinant of biomass. The ability to select for improved photosynthesis within modern bread wheat cultivars is constrained by genetic 'bottlenecks' which occurred following domestication. To increase genetic diversity it may be possible to exploit wild relative germplasm, of particular interest are the genus' Aegilops and Triticum, which exhibit variable photosynthetic traits. In this work, double haploid lines of bread wheat containing introgressions from *Aegilops mutica* and *Triticum urartu* are to be screened by growing multiple lines at sub-ambient CO_2 (~ 200 ppm) in a controlled environment chamber. Past studies used CO_2 to select for improved photosynthesis by screening below the compensation point, based on the hypothesis that any surviving or 'high performing' plants must exhibit a lower compensation point and therefore exhibit an improved photosynthetic rate and / or decreased photorespiration and respiration. Enhanced abiotic stress tolerance not related to compensation point could also be a factor. Growth of the double haploid lines at sub-ambient CO_2 and measurements of photosynthetic parameters will allow us to identify 'high performing lines' and establish the physiological and/or biochemical mechanisms that underpin improved photosynthesis in these lines.

POSTER 8

Identifying molecular sequence changes in C_4 photosynthesis associated genes using natural variation

Michael Niklaus[1], Kelly S[1]

1. Department of Plant Sciences, University of Oxford, UK

C_4 and CAM photosynthesis are evolutionary adaptations of C_3 photosynthesis. Interestingly, the C_4 pathway evolved at least 62 times independently from ancestral C_3 photosynthesis. Analysis of positive selection within genes from species that do C_4 photosynthesis has provided insights into molecular sequence changes associated with C_4 photosynthesis. However, these analyses may overlook functional sites in genes whose rate of change relative to the rate of synonymous change is not high. For example, a site in a protein sequence that is valine in all C_3 plants but is serine in all C_4 plants is a potential key biochemical difference between C_3 and C_4 plants irrespective of the rate at which that change occurred in the protein sequence. Therefore, an alternative approach that is rate independent and that can consider a broader range of changes could provide new insight into the molecular evolution of C_4 photosynthesis.

Here a comparative sequence analysis of thirty C_4 and seventeen C_3 species representing eighteen independent evolutionary origins of C_4 photosynthesis is presented. The analysis identified 14 genes closely linked to the C_4 cycle that contained amino acid sites that were significantly different between C_3 and C_4 species. This set included 12 sites in PEPC, two of which correspond to sites previously shown to be important determinants of the C_4 function of PEPC in *Flaveria trinervia* providing experimental validation of the approach. A further four sites in PEPC were also previously identified as under positive selection in C_4 grasses. The remaining 6 sites have not previously been linked to C_4 function and thus this novel approach presented here has the potential to provide new information about the molecular evolution of C_4 photosynthesis.

POSTER 9

Quantifying light tolerance and photoprotection in spring ephemerals

Sam Wilson[1], Ruban AV[1]

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Spring ephemerals are widely distributed throughout temperate ecosystems. Two of these ephemerals, *Berteroa incana* and *Sisymbrium altissimum* of the Brassicaceae family, are suspected to be capable of tolerating high-light intensities. Whilst their macroscopic physiological adaptations have been well characterised, the interplay between photoinhibition and photoprotection on a molecular level has not been fully explored. Using a novel chlorophyll fluorescence methodology, the fraction of functional photosystem II (PSII) reaction centres (RCII) can be accurately quantified non-destructively *in vivo*, alongside photoprotective strategies. This is achieved through estimation of the redox state of QA, with the parameter of photochemical quenching in the dark (qPd). From this, the effects of reversible photoprotection, NPQ, and chronic photoinhibition can be disentangled, and the reduction of the quantum yield of PSII can be more accurately explained. Thus, the onset of photoinhibition can be measured to quantify the light tolerance of PSII and protective NPQ (pNPQ) for each plant. Within spring ephemerals acclimated to both low- and high-light intensities, the kinetics of both the decline and recovery of the number of functional RCIIs have been accurately determined, in parallel with pNPQ and light tolerance measurements. These kinetics have been modelled and further characterised in order to explain the increased light tolerances of both *Berteroa* and *Sisymbrium*.

POSTER 10

CyanoGate: A Golden Gate modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax

Grant Gale[1,2,3], Vasudevan R[1,2], Schiavon AA[1,2], Wang B[2], Howe CJ[3], Lea-Smith D[4], McCormick AJ[1,2]

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Cyanobacteria are ancient photoautotrophic prokaryotes that are considered to be the ancestral source of chloroplasts in higher plants. The ability to synthesise complex molecules using captured light energy and fixed atmospheric carbon dioxide has made cyanobacteria a promising platform for the renewable production of high value chemicals. Several new tools and parts are currently being developed for cyanobacteria to advance metabolic engineering to levels commensurate with other model cell factories (e.g. yeast, *E. coli*). We have developed a cloning system which includes a suite of new vectors and modular parts called CyanoGate, which is compatible with the syntax of the Golden Gate Modular Cloning (MoClo) Toolbox for plants. Here we present 33 synthetic promoters and 5 native promotors, one new origin of replication, transformation plasmids for chromosomal integration or self-replication, and CRISPR interference tools for gene repression. We have characterised these parts in two separate cyanobacterial species, *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* UTEX 2973. Developing new molecular tools in this common syntax will maximise community access and impact as well as facilitate ease of sharing and integration into current libraries for use alongside existing parts.

POSTER 11

Feeding Rubisco with CO_2: modifying chloroplastic carbonic anhydrase activity in *Arabidopsis thaliana* for introducing an algal CO_2-concentrating mechanism

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The efficiency of CO_2 assimilation is a major bottleneck that limits productivity in C_3 crops. This is primarily a result of the catalytic properties of Rubisco, underlying a competitive relation between two of its substrates, CO_2 and O_2. Assimilation of O_2 instead of CO_2 by Rubisco leads to net carbon and nitrogen losses and reduces photosynthetic efficiency, in an energetically wasteful process termed photorespiration. The operating efficiency of Rubisco in C_3 crops could be increased by introducing a CO_2-concentrating mechanism (CCM). CCMs are found in several photosynthetic organisms and act to increase the local CO_2 concentration around Rubisco, thus reducing photorespiration. In biophysical CCMs, such as the one found in the green alga *Chlamydomonas reinhardtii*, inorganic carbon is actively pumped into the cell and into a Rubisco-containing compartment called the pyrenoid. There, it is converted into CO_2 by a specialised carbonic anhydrase (CA) called CAH3. Appropriate CA activity in the chloroplast is critical for the functionality of the Chlamydomonas CCM. We aim to progress current work to reconstitute an algal CCM in the C_3 plant model organism *Arabidopsis thaliana* by i) correctly localising CAH3 to higher plant chloroplasts, and ii) removing potentially competitive native carbonic anhydrase activities in the chloroplast. Work is underway to generate a knock-out (KO) line for all chloroplastic CAs. While removal of the majority of chloroplastic CAs does not a negatively affect plant growth, deletion of *βCA5* (*AT4G33580*) has a severe impact on growth and fecundity. Therefore, we are currently investigating the reason for this phenotype and different strategies to rescue the *βca5* mutant.

POSTER 12

Characterising WHIRLY1 functions in plastids

Rachel Taylor[1], Rouster J[2], Foyer CH[1]

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WHIRLY1 is a member of a small family of single stranded DNA-binding proteins with multiple functions in plants. It is dual located to both plastids and nuclei. In the nucleus, WHIRLY1 modulates telomere length and functions as a transcriptional activator of senescence- and pathogenesis-related genes. WHIRLY1 functions in chloroplasts appear to vary between species. For example, in Arabidopsis chloroplasts, WHIRLY1 functions with other proteins to control genome stability, while in barley this protein is implicated in the regulation of plastid gene expression and WHIRLY1-deficient lines had a delayed greening phenotype. Moreover, WHIRLY1 was shown to be required for ribosome formation in maize chloroplasts; loss of the protein resulted in a severe albino phenotype, although this has not been described in WHIRLY1-deficient Arabidopsis or barley leaves. Hence, many of the roles of WHIRLY1 in plastids remain uncertain, requiring a deeper characterisation. Arabidopsis has two closely related WHIRLY proteins in the chloroplasts, WHIRLY1 and WHIRLY3. Mutant lines deficient in either or both proteins are currently under investigation. This study will describe the phenotypes of these lines, together with a detailed characterisation of the differences in shoot phenotypes between species. The potential roles of the WHIRLY proteins in the regulation of photosynthesis, leaf senescence and stress tolerance will also be discussed. Transgenic wheat and maize lines with altered expression of WHIRLY1 are currently in production, with a view to enhancing stress tolerance and ultimately increase yields in these important crop plants.

POSTER 13

The role of catalase enzyme subcellular localization in plant development and stress responses

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1. School of Molecular and Cellular Biology, University of Leeds, UK

Reactive oxygen species (ROS) play a key role in both stress responses and developmental pathways in all living organisms. The level of ROS molecules is controlled by different antioxidant enzymes as well as non-enzymatic antioxidants. Peroxisomal catalases are considered as one of the key antioxidant enzymes implicated in the detoxification and defensive mechanisms against generated ROS particularly hydrogen peroxide (H_2O_2) molecules which are generated in peroxisomes as a result of photorespiration and β-oxidation. In Arabidopsis, catalase exists in three isoforms of which the catalase 2 (CAT2) is the most crucial for photorespiration; the *cat2-1* mutant is a photorespiratory mutant and several studies have been conducted to investigate the phenotype of *cat2-1* mutant lines.

Catalase is targeted to peroxisomes by the PTS1 import receptor, but its C terminal targeting signal does not match the normal PTS1 consensus. In Arabidopsis there is an alternative splicing event that alters the C terminal amino acid sequence of catalase and therefore potentially alters catalase location. Transgenic lines in which the *cat2-1* mutant has been transformed with the wild type *CAT2* gene, the alternative splice variant which is predicted to be cytosolic, and a version that has a consensus PTS1 sequence ARL at the C terminus, all under the control of the native *CAT2* promoter have been selected. Molecular and phenotypic characterisation is in progress and will be presented. In addition, transgenic lines with YFP-tagged wild type, alternative splice variant and ARL variant being selected to verify subcellular localisation.

These data will give insight into the mechanism of targeting of catalase and the function of the presumed cytosolic splice variant.

POSTER 14

Chloroplast development in barley leaves is controlled by WHIRLY1

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The regulation of chloroplast biogenesis requires coordinated expression of genes localised in the plastome and nuclear genomes. The single-stranded DNA binding protein, WHIRLY1, which is localized in the chloroplasts and nucleus of the same cell has important but poorly characterised roles in this process. We therefore investigated the functions of WHIRLY1 in the leaves of wild type barley and two transgenic lines (W1-1 and W1-7) that have less than 5% of the wild type WHIRLY1 protein. The greening of the developing leaves was delayed in the WHIRLY1-deficient seedlings relative to the wild type. However, the primary leaves of seedlings of all lines reached a similar stage of development within 14 days after germination. At earlier stages, the WHIRLY1-deficient seedlings had significantly lower levels of chlorophyll than the wild type. Chlorophyll a fluorescence quenching analysis revealed that the establishment of photosynthesis was delayed in the WHIRLY1-deficient leaves. The levels of nuclear-encoded chloroplast transcripts and proteins were significantly higher in the WHIRLY1-deficient leaves than the wild type. In contrast, the levels of transcripts and proteins encoded by the plastome were significantly lower in the wild type than the WHIRLY1-deficient seedlings, despite the fact that the WHIRLY1-deficient leaves. The W1-1 and W1-7 leaves had double the amount of plastid DNA (ptDNA) than the wild type, both at the early stages of development (7 days) and in green (3 week-old) leaves. The WHIRLY1-deficient seedlings showed aberrant splicing of plastid rRNAs (23S rRNA) and were deficient in plastid ribosomal proteins. These results demonstrate that WHIRLY1 is required for the development of fully functional chloroplasts but only at the early stages of leaf development. Moreover, WHIRLY1 is required for plastid ribosome production, as well as the transcription of chloroplast-encoded genes. Lastly, chloroplast to nucleus signalling is also impaired in the absence of WHIRLY1.

POSTER 15

Bicarbonate enrichment of chloroplasts *via* the mammalian anion exchanger AE1

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Bioengineering carbon-concentrating mechanisms (CCMs) into C_3 plant species has been predicted to substantially increase the photosynthetic efficiency and yield of crops in order to secure future global food security. Elevating the CO_2 concentration around RubisCO aims at favouring enzymatic carbon fixation at the expense of photorespiration. Cyanobacteria achieve this by localizing Rubisco to specialized subcellular microcompartments termed carboxysomes, which contain carbonic anhydrase and a specialized faster RubisCO form.
In collaboration with the Parry lab from Lancaster University and the Hanson lab from Cornell University we are working on introducing cyanobacterial carboxysomes into chloroplasts of tobacco. Along with the carboxysome components, a truncated form of the human anion exchanger AE1 will be expressed in the inner envelope of chloroplast to facilitate Cl^-/HCO_3^- exchange, thus enhancing the HCO_3^- concentration in the chloroplast stroma. The functionality of AE1 will be verified with pH-sensitive fluorophores that are targeted to the chloroplast membrane and stroma.

POSTER 16

Determining the molecular and metabolic mechanisms that underpin the blackening phenomenon in cut carrots

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Carrots are a major root vegetable crop in the UK, with over 700,000 tonnes being produced in the UK each year. Freshly harvested carrots are distributed to nearly every shop in Britain on almost every day of the year, however problems can arise with cut carrot products harvested in later months. In particular, the shelf life of carrot products can be shortened because they exhibit an unpredictable tendency to blacken, *via* mechanisms that are as yet unknown. All affected bags of carrots have to be thrown away, causing food waste and commercial loss. As a first step to identifying the metabolic and molecular mechanisms that cause blackening in cut carrots, samples of control and black areas of cut carrots were isolated and subject to metabolic profiling using HPLC and Mass Spectrometry. The samples that showed blackening have similar levels of carotenoid pigments but altered levels of primary and secondary metabolites, particularly the pools of some amino acids. These data will be discussed in terms of a switch from primary to secondary metabolism that occurs in the black areas of the cut carrots.

POSTER 17

The search for the elusive thylakoid membrane bicarbonate transporter of *Chlamydomonas reinhardtii*

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Chlamydomonas reinhardtii induces a CO_2 concentrating mechanism (CCM) that turbocharges photosynthesis under low CO_2 conditions (<400 ppm CO_2). This mechanism is supported by a series of bicarbonate transporters shuttling external inorganic carbon towards the pyrenoid, a Rubisco rich organelle. Interestingly, the current CCM model requires an unidentified thylakoid membrane bicarbonate transport component which shuttle bicarbonate from the chloroplast stroma into the thylakoid lumen.

We find that a mutant in a bestrophin-like protein identified through a recent CCM protein-protein interaction study, suffers a growth defect under low CO_2, high pH conditions which promotes a high HCO_3^-:CO_2 ratio. Using homology modelling, we find that the residue pattern at the channel pore lining as well as the electrostatic potential mimic chicken bestrophin, a homologue of a bicarbonate permeable channel. Preliminary microscopy screening shows this phenotype does not exhibit a drastic change in pyrenoid assembly and starch sheath formation. Together with the localisation of other Chlamydomonas bestrophin-like family members at the thylakoid membrane surrounding the pyrenoid periphery, we postulate that the pH dependent high-CO_2 phenotype is due to defective bicarbonate transport across the thylakoid membrane, leading in failure to establish a fully functional CCM.

POSTER 18

Investigating the regulation of NADP-malic enzyme in C_4 monocot grasses during dark–light transitions

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NADP-malic enzyme (ME) catalyses the decarboxylation of malate, requires a divalent metal ion as cofactor, and is localised in the chloroplast of bundle sheath cells in C_4 plants. In NADP-ME subtype C_4 plants malate is the predominant translocated C_4-acid, though aspartate may contribute to 10–15% of the assimilated carbon pool. To study the regulation of NADP-ME in response to pH and illumination, we harvested leaves of C_4 plants (*Setaria viridis*, *Sorghum bicolor* and *Zea mays*) grown in a controlled environment in specific light/dark periods, from which protein extracts were subjected to mass spectrometry analyses and *in vitro* biochemical characterisation to determine NADP-ME pH optimum and Michaelis-Menten kinetics. We report novel phosphorylation of NADP-ME with the advantage of an ultra-sensitive mass spectrometer (Orbitrap, LC-MS/MS) and differences to the NADP-ME kinetic properties from darkened and illuminated leaves of these C_4 grasses and the corresponding shift in their pH optimum will be reported in detail for the first time. These results also show that the classification of C_4 plants as distinct subtypes may be an oversimplification and may not accurately reflect the regulation of carbon flux, as interspecies differences in the catalytic properties of NADP-malic enzyme seem to exist. Together, these latest findings add to our understanding of the regulation of NADP-ME in C_4 plants, thereby advancing the wider objective of engineering the C_4 trait into important C_3 crops.

POSTER 19

Plantae.org: a place to connect, curate, collaborate, and co-create

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Plantae is the global community and knowledge hub for plant scientists. It was created to bring together the plant science community as a strong collective presence and voice. Plantae is for everyone in the plant science community regardless of location or career phase, from plant science students to educators and researchers to authors, editors, and professionals. Through Plantae, you can follow individuals, join networks, find resources, contribute to collections, and identify collaborators. Early-career scientists can find opportunities to increase their visibility within the community, for example by writing blog posts, curating article collections and managing discussions. You'll also find co-created projects including webinars (topics range from *Using WordPress* to *Computational Plant Science*), the Taproot Podcast series, a weekly plant science research roundup, and a plant science focused job board. Drop by to learn how Plantae.org can help you advance your career.

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Jesica Asagwara	Newcastle University
Nicky Atkinson	University of Edinburgh
Jessica Bampton	University of Southampton
Sarah Blackford	Lancaster University
Laura Briers	University of Nottingham
Alexandra Burgess	University of Nottingham
Elizabete Carmo-Silva	Lancaster University
Rona Costello	University of Oxford
Gustaf Degen	Lancaster University
Tom Emrich-Mills	University of York
CC Foo	University of Nottingham
Monika Gajecka	University of Silesia in Katowice
Grant Gale	University of Edinburgh
Emmanuel Gonzalez-Escobar	Lancaster University
Ciaran Griffin	University of Plymouth
Jonathan Griffin	Lancaster University
Ross-William Hendron	University of Oxford
Binquan Huang	University of Oxford
Ivan Jauregui	Lancaster University
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