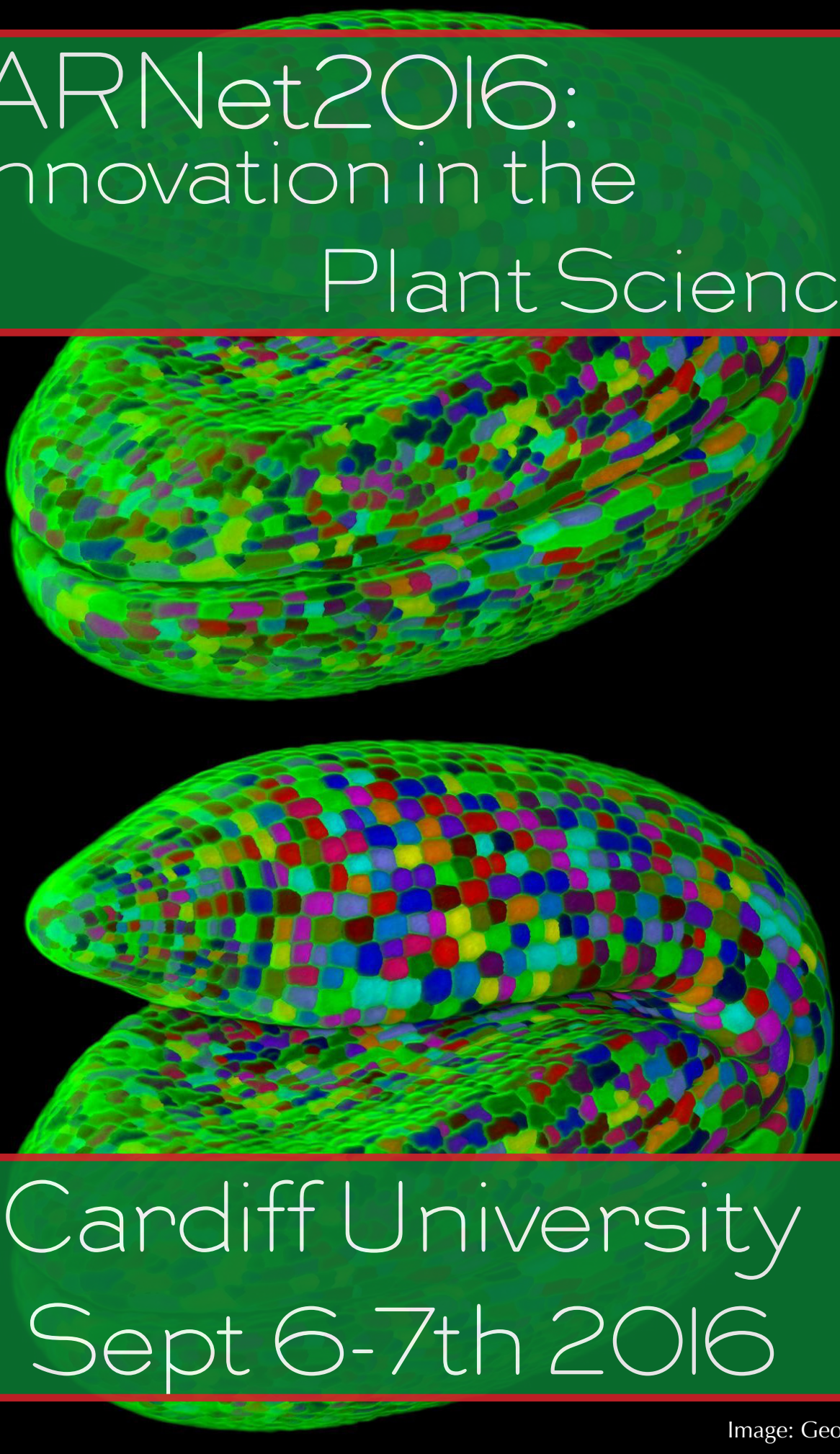
GARNet2016:
Innovation in the Plant Sciences



Cardiff University
Sept 6-7th 2016

Image: George Bassel

Welcome

The GARNet Advisory Committee are delighted to welcome you to "GARNet2016: Innovative in Plant Sciences". Every two years we aim to organise a meeting that captures exciting developments in plant science, with a focus on UK research capability. We are grateful for continued BBSRC funding until 2020 and this meeting is part of our committment to support the uptake of novel technologies. Hopefully the scientific sessions represent GARNet's efforts to encourage researchers to consider translational aspects of their research, whilst making clear our support of fundamental research that requires using the incredible breadth of resources available across the plant sciences.

By organising three workshop sessions and providing ample opportunity for younger researchers to present their work we hope to faciliate the scientific development of attendees.

The GARNet Advisory Committee

Katherine Denby (University of York)
Antony Dodd (University of Bristol)
Nicholas Harberd (University of Oxford)
Ian Henderson (University of Cambridge)
Saskia Hogenhout (John Innes Centre)
Sabina Leonelli (University of Exeter)
Sean May (NASC, University of Notttingham)
Jim Murray (Cardiff University, GARNet PI)
Christine Raines (University of Essex)
David Salt (University of Nottingham, GARNet Chair)
Steven Spoel (University of Edinburgh)
Zoe Wilson (University of Nottingham)

The core GARNet team of Geraint Parry and Ruth Bastow are based at the Cardiff University.

For more information please see the GARNet website at www.garnetcommunity.org.uk, and the GARNet blog at http://blog.garnetcommunity.org.uk.
You can also follow us on Twitter on @GARNetweets

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Thanks

Gold Exhibitors

Booklet Contributors

Meeting Information

Please see facing page for Meeting locations

Connectivity:
The Haydn Ellis Building is serviced by the EduRoam network but if you need a guest ID then please ask at registration

Catering:
The coffee breaks, lunch and the evening buffer dinner will be held in the Haydn Ellis Foyer. If you have informed us of certain dietary requirement then those meals will be labelled.

Poster Session and Evening Reception:

During the poster session please enjoy a glass of wine and be sure to visit the sponsor booths. Most conferences would not be possible without exhbitor's generous support so we encourage you to interact with our sponsors.

Evening Event:

Your conference bag contains a drink token for an evening event that takes place in the ZeroDegrees bar on Westgate Street. There will be a coach provided to transport up to 50 attendees to the venue. For those that choose to walk, it will take 20-25 minutes that will allow the opportunity to wander past Cardiff Castle and the Principality Stadium.

Members of Jim Murray's lab will help with directing attendees to the venue but please also refer to the location information.

Please bare in mind when leaving the evening event that Cardiff is a big city so the usual care needs to be taken when returning to your accommodation.

Taxis Numbers:

Premier Taxis: 029 2055 5555
Dragon Taxis: 029 2033 3333

Social Media:

We encourage you to share the meetings exciting science with the wider community so the twitter hashtag is #GARNet2016

GARNet2016: Innovation in the Plant Sciences

Meeting Information

Meeting Venue: Haydn Ellis Building, Maindy Rd, Cardiff, CF24 4HR

Evening Event: Zerodegrees, 27 Westgate Street, Cardiff, CF10 1DD

Travel and Accommodation

Cardiff Central Railway Station, Central Square, Cardiff CF10 1EP
Cathays Railway Station, Park Place, Cardiff

Senghennydd Hall, Senghennydd Rd, Cardiff CF24 4AG
Cardiff Hilton, Kingsway, Cardiff, CF10 3HH

Brief Program

Tuesday September 6th

From 8am: Registration Open and Poster setup

9am: Pre-Meeting Workshop: 'Finding your Arabidopsis Gene in Wheat'

11am: Session 1: Frontiers in Plant Imaging

12.50pm: Lunch

1.50pm: Session 2: Enabling the Translational Pipeline

3.15pm: Coffee Break

3.45pm: Session 3: Plant Synthetic Biology

5.15pm: Flash Presentations

6.15pm: Poster Session

8pm: Evening Event

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Brief Program

Wednesday September 7th

9am: Session 4: Genomics Tools for Gene Discovery

11am: Coffee Break

11.30am: Concurrent Workshops
- Usage and Application Development within Araport
- Introduction to CRISPR-Cas: troubleshooting and target design

1pm: Lunch

2pm: Session 5: Cell Signalling

4pm: Meeting End

Detailed Program

Tuesday September 6th

From 8am: Meeting Registration and Poster setup *Haydn Ellis Building Foyer*

9am - 10.30am: Pre-Meeting Workshop: Finding your Arabidopsis Gene in Wheat. Organisers: Philippa Borrill, Nikolai Adamski and Cristobal Uauy (John Innes Centre) *Lecture Theatre*

Session 1: Frontiers in Plant Imaging *Lecture Theatre*

11am: Meeting Welcome: Jim Murray, GARNet PI, Cardiff University

11.05am: Opening Plenary:
Ben Scheres (Wageningen University): PLETHORA transcription factors and the master regulator concept

11.50am: Jens Tilsner (University of St Andrews): Improving genetically encoded fluorescent plant RNA sensors

12.10pm: Malcolm Hawkesford (Rothamsted Research): Automated and high throughput imaged-based field phenotyping

12.30pm: Darren Wells (University of Nottingham): Imaging the hidden half: phenotyping using X-ray tomography

12.50pm: Lunch *Foyer*

Session 2: Enabling the Translational Pipeline *Lecture Theatre*

1.50pm: Jim Murray (Cardiff University): A plant scientist led astray: Confessions of a scientific wayfarer

2.10pm: Mike Roberts (University of Lancaster): Exploiting plant defence priming for sustainable crop protection

2.30pm: Neil Bruce (University of York): Defusing the environment: engineering plants to remediate explosives pollution

2.50pm: Discussion Session to highlight opportunities for translation in plant science. Chair: Jim Murray
Jayne Brookman, Head of Agrifood, KTN
Jon Wood, Innovate UK

3.15pm: Coffee Break *Foyer*

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Detailed Program

Tuesday September 6th

Session 3: Plant Synthetic Biology *Lecture Theatre*

3.45pm: Cathie Martin (John Innes Centre): How to overcome constraints on metabolic engineering for effective synthetic biology in plants

4.15pm: Christine Raines (University of Essex): Re-designing photosynthesis to increase yield

4.35pm: Anil Day (University of Manchester): Applications of synthetic biology in chloroplasts

4.55pm: Nicola Patron (The Earlham Institute, Norwich): Foundational technologies and standards for engineering plant biology

5.15pm: Flash Presentations: 'Two Minutes, Two Slides, No Waiting'

6.15pm: Poster Session with buffer dinner *Foyer*

8pm: Evening Event with drinks and buffet. at **ZeroDegrees**, Westgate Street. *Cardiff City Centre*

Wednesday September 7th

Session 4: Genomics tools for Gene Discovery *Lecture Theatre*

9am: Chris Town (Araport): Araport: a 21st century platform for Arabidopsis data integration

9.30am: David Marshall (James Hutton Institute): Software tools and technologies for exploring germplasm diversity

9.50am: Katherine Denby (University of York): iPlantUK: computational resources for plant scientists

10.10am: Marnix Medema (Wageningen University): Computational genomic discovery of plant biosynthetic pathways

10.30am: Discussion of future resources for analysis of big data in the Plant Sciences. Chair: Katherine Denby

11am: Coffee Break *Foyer*

Detailed Program

Wednesday September 7th

11.30am: Concurrent Workshop Sessions:

Workshop 1: Usage and Application development within Araport.
Organiser: Chris Town and Sergio Contrino *Seminar Room*

Workshop 2: Introduction to CRISPR-Cas, troubleshooting target design and verification of mutants.
Organisers: Vladimir Nekrasov and Amanda Hopes *Lecture Theatre*

1pm: Lunch *Foyer*

Session 5: Cell Signaling *Lecture Theatre*

2pm: Stefan Kepinski (University of Leeds): Growth angle control in higher plants
2.20pm: Eirini Kaiserli (University of Glasgow): Signal Integration in Nuclear Microdomains
2.50pm: Daniel Gibbs (University of Birmingham): Oxygen and nitric oxide signalling through functionally diverse targets of the N-end rule pathway

Final Plenary:
3.10pm: Niko Geldner (University of Lausanne): The endodermal barrier - walking the thin line between uptake and protection.

4pm: Meeting End

PLETHORA transcription factors and the master regulator concept

Ben Scheres, Luca Santuari, Du Yujuan, Gabino Sanchez-Perez, Renze Heidstra

Wageningen University Research, The Netherlands

In Arabidopsis roots, the phytohormone auxin and six redundantly acting PLETHORA transcription factors control many aspects of developmental progression. Coordination of this progression defines zones of cell division, cell expansion and cell differentiation. We combined experiments and computational modelling to unravel a dynamic interplay between auxin and the PLT proteins. High and prolonged auxin concentrations generate a narrow PLT transcription domain, and a PLT protein gradient extends outward from this domain exploiting growth dilution and cell-to-cell movement. Different PLT levels define two distinct meristem zones and the expansion/differentiation boundary (Mähönen et al, 2014).

How can a transcription factor gradient encode properties such as stemness and differentiation? We have approached this question by investigating the direct and indirect targets of PLT transcription factors using induced expression and ChIP-seq approaches. The results indicate that division and differentiation control through PLT transcription factors can be separated at the level of induced and repressed target genes. Another way to test the full requirement of a set of redundant transcription factors is to find ways to eliminate the activity of all members in a subset of meristems.

We have achieved this for the first time by exploiting the cross-dependency of PLT genes during lateral root development and present data on primordium development without PLT regulators suggesting that, in line with its transcriptional target set, PLT activity is strictly required for apical-basal and radial patterning of lateral root primordia.

Mähönen et al., Nature 515, 125-129, 2014.

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Session I: Imaging

Improving genetically encoded fluorescent plant RNA sensors

David Burnett[1], **Jens Tilsner**[1,2]

1- University of St Andrews
2- The James Hutton Institute

The localisation of RNA virus genomes and mRNAs within cells provides important information about RNA processing and mechanisms of viral infection. Several techniques have been developed that allow dynamic, live cell imaging of specific RNAs but many require tagging of the RNA of interest with multiple (up to 96) tandem copies of stem-loop forming sequences (Tilsner, 2015). This can severely affect localisation, stability and translation of tagged RNAs and is particularly unsuitable for viruses, which usually do not tolerate such extensive secondary structure.

The sequence-specific RNA binding Pumilio homology domain (PUMHD) can be engineered to recognise an RNA of choice with sub-nanomolar affinity. These properties have enabled subcellular tracking of viral RNAs in plant cells (Tilsner et al., 2009, 2013). For this purpose, two PUMHDs are engineered to bind closely adjacent target sequences within the viral genome, and fused to halves of a split fluorescent protein (PUMHD-bimolecular fluorescence complementation; PUM-BiFC). Whilst PUM-BiFC has been successfully applied to several different plant viruses, there remain limitations to its specificity and sensitivity, in particular due to non-specific/permanent reconstitution of fluorescent complexes.

We have made several modifications to PUM-BiFC to improve its signal-to-noise-ratio. Additionally, we have developed a new live cell RNA imaging system based on a sequence-specific RNA binding protein with picomolar affinity, which enables more sensitive imaging of plant RNA virus genomes compared to PUM-BiFC.

Tilsner J, et al. 2009. The Plant Journal 57: 758-770.
Tilsner J, et al. 2013. Journal of Cell Biology 201: 981-995.
Tilsner J. 2015. Journal of Microscopy 258: 1-5.

Automated and high throughput imaged-based field phenotyping

Malcolm Hawkesford, Pouria Sadeghi, Nicolas Virley, Kasra Sabermanesh, Andrew Riche, Adam Michaelski

Rothamstead Research

Two approaches for field phenotyping are being employed for wheat germplasm evaluation in the field, one based on UAVs for extensive plot trials, and another ground-based robotic installation for intensive evaluation in a limited area.

The UAVs are used to simultaneously collect thermal infrared, multispectral and conventional RGB images. The thermal imagery is indicative of drought stress. The multispectral imaging is used to calculate NDVI data for estimating percentage ground cover and canopy colour changes, e.g. senescence or canopy health. The RGB images are used to produce digital surface models, from which crop height data can be extracted. Many thousands of plots can be evaluated within a short period of time with resolution at the field plot level.

A programmable, automated robotic platform (developed with Lemnatec GmbH, Aachen, Germany) with a dedicated sensor array, accurately positionable in three dimensions and mounted on a fixed trackway has been developed to facilitate 24 h continuous monitoring of plant and crop performance and health throughout the entire season. Sensors comprise a high definition RGB camera, two hyperspectral cameras covering 400-1000 nm and 550-1700 nm ranges, a thermal camera, a system for imaging chlorophyll fluorescence (CropReporter, Phenovation Life Sciences) and twin scanning lasers for 3-D information capture. Image analysis with high temporal, spatial and spectral resolution enables identification of plant organs and facilitates specific growth measurements and identification of key growth stages.

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Session I: Imaging

Imaging the hidden half: phenotyping using X-ray tomography

Darren Wells

University of Nottingham

X-ray Computed Tomography (X-ray CT) is a non-invasive, non-destructive imaging technique for visualisation and quantification of the interior structure of samples in three dimensions based on the attenuation of X-rays. Recent advances in tomographic imaging now permit the use of CT to phenotype root systems in soils, revealing the hitherto hidden half of plants.

The Hounsfield Facility at Sutton Bonington was established to develop CT techniques for imaging biomaterials with a focus on rhizosphere research and plant and soil phenotyping. Current projects in the Facility will be reviewed, including phenotyping of adaptive traits in model species, root system architectures of crops, and canopy imaging. An evaluation of the utility of CT against alternative technologies will be presented.

A plant scientist led astray: Confessions of a scientific wayfarer

Jim Murray

Cardiff University

Over twenty years ago, chance funding led to the starting of a new research project on firefly luciferase in my lab. Ten years later, this led on to the co-founding of the spin-out company Lumora with Dr Laurence Tisi to exploit a technology that we had developed, which reports on DNA or RNA amplification through a real-time light output.

I will describe the journey of the plant scientist learning to become something of an entrepreneur, leading to the launch of products developed by Lumora through global licensee 3M, the winning of BBSRC Commercial Innovator of the Year in 2012 by Murray and Tisi, and the acquisition of Lumora by Erba Mannheim in 2015

http://www.erbamdx.co.uk/news/175-erba-diagnostics-mannheim-acquires-lumora

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Session 2: Translation

Exploiting plant defence priming for sustainable crop protection

Mike Roberts

University of Lancester

Much of crop protection in the last century has relied on the application of pesticides which are directly toxic to the pest or pathogen. However, there are now strong drivers to adopt alternative crop protection technologies that are both environmentally- and consumer-friendly. Plants possess a wide range of natural defence mechanisms which together contribute to pest and disease resistance. Some of these include permanent physical and chemical barriers to attack, whilst others are induced only in response to infection or herbivore feeding. There are several well-known plant defence systems that control induced resistance against microbial pathogens and invertebrate pests. Each of these forms of resistance includes the *de novo* production of defensive proteins and metabolites, but also acts to 'prime' cells such that they are able to respond more rapidly to future attack. We have shown that molecules that are able to activate induced resistance when applied to plant tissues (including some key plant hormones) can also be used to establish long-term priming of plant defence. In particular, we have shown that treatment of seeds or seedlings with such compounds can generate long-lasting improvements in pest and disease resistance in a range of crop species by priming natural plant defence responses. Priming improves resistance with minimal impacts on plant productivity, and is compatible with other biological and chemical control measures. I will describe the process for commercial application of such a priming treatment and outline our current understanding of the mechanisms for long-term defence priming.

Defusing the environment: engineering plants to remediate explosives pollution

Neil Bruce

CNAP, University of York

Explosive compounds used in munitions are highly toxic and the potential for progressive accumulation of such compounds in soil, plants and groundwater is a significant concern at military sites. It is estimated that in the U.S. alone 10 million hectares of military land are contaminated with components of munitions. Explosives pollution is, however, a global problem with large amounts of land and ground water contaminated by TNT and RDX, including polluted sites in the U.K. dating back to the First and Second World Wars. There is a pressing need to develop sustainable in situ technologies to contain and treat these pollutants.

Plants have a remarkable ability to extract compounds from their environment and have evolved complex signalling and enzyme systems to deal with a diverse range of toxic chemicals. We have, therefore, been uncovering the molecular mechanisms behind these detoxification processes in plants and have used this knowledge, in combination with studies on the bacterial degradation of explosives, to successfully engineer transgenic plants able to remediate toxic explosive pollutants. Our engineered transgenic plant systems can efficiently remove toxic levels of TNT and RDX from contaminated soil and water. As a result of our advances in knowledge of the biodegradation of explosives, we have developed transgenic switchgrass lines that are currently being tested in field trials in the U.S.

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Notes

How to overcome constraints on metabolic engineering for effective synthetic biology in plants

Cathie Martin

The John Innes Centre

I will talk about how to achieve effective metabolic engineering of polyphenol levels in plants. Such engineering can lead to new production systems for high value chemicals from plants.

Polyphenol-enriched foods can also be used to assess the benefits of these metabolites in the diet, through the new field of comparative nutrition, and offer the possibility of biotech food products that protect consumers from chronic conditions.

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Session 3: Synthetic Biology

Re-designing photosynthesis to increase yield

Christine Raines

University of Essex

It has been estimated that over the next four decades a 50% increases in the yield of grain crops such as wheat and rice will be required to meet the global demands for food and fuel.

Traditionally single gene constructs have been used to add, alter or remove individual steps in a pathway but the advent of multigene cloning approaches now allows for simultaneous manipulations of multiple targets.

We have developed a transformation pipeline to produce a large number of transgenic tobacco and Arabidopsis plants with altered complements of enzymes and proteins involved in the process of photosynthesis.

Results from the analysis of these plants have demonstrated the potential of multigene-stacking to improve photosynthesis and plant yield, with future applications in the generation of higher yielding crop plants for the production of biofuels and food.

Applications of synthetic biology in chloroplasts

Anil Day

University of Manchester

Chloroplasts are members of the plastid family of organelles, which are important metabolic compartments in plant cells. Plastid transformation opens up a route for engineering plastid genomes for synthetic biology applications. Angiosperm plastid genomes encode around 100 genes, are relatively small (~150 kb) and present in multiple copies per cell. Precise manipulation of the plastid genome is facilitated by the predominance of homologous recombination in this organelle.

Conventional cloning methods are largely responsible for designing and building plastid transformation vectors. Isolating plastids with new functions is empowered by the capacity of a eubacterial-like transcriptional/translational apparatus in this organelle which enables multiple foreign proteins to be expressed from synthetic operons. User designed plastids range from those programmed to express single proteins to more challenging metabolic engineering projects requiring multiple proteins, for example, introducing nitrogen fixation genes into plastids. Identifying the sequences required for the replication and maintenance of plastid genomes provides a route towards designing and assembling synthetic plastid genomes.

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Session 3: Synthetic Biology

Foundational technologies and standards for engineering plant biology

Nicola Patron[1,2,3]

1- The Earlham Institute
2- The John Innes Centre, Norwich Research Park, NR47UH, UK
3- OpenPlant Consortium: The University of Cambridge, The John Innes Centre, The Sainsbury Laboratory and The Earlham Institute.

Synthetic biology is the application of engineering principles to the design and modification of biological systems. This nascent field has established biological standards that define ways in which DNA parts can be assembled together using the same reagents and protocols and aims for libraries of characterized biological parts and devices that behave predictably.

A few years ago, the major bottleneck was the construction of complex DNA molecules. One step parallel assembly methods, now being applied at the genome scale, have propelled us over this hurdle. The Earlham DNA Foundry provides an automated platform that assembles standardised DNA parts (Phytobricks) in a defined Plant Common Syntax at nanoscales and we are adding capabilities to automate delivery to plant cells.

Our research makes use of the Foundry's capabilities and pursues the rational design of minimal synthetic regulatory elements by understanding promoter architecture. We are also improving our genome engineering tools for plants, increasing the efficiency and specificity of programmable nucleases and expanding the genome space accessible for engineering. We are applying our tools to the production of transgene-free, engineered crops and aiming for bespoke plant chassis, genetically tailored for the biosynthesis of valuable natural products and proteins.

Flash Presentations

- A Beginner's Guide to Wheat – www.wheat-training.com
Nikolai Adamski: The John Innes Centre
Poster: 01

- Gene expression patterns unlocked by a species-agnostic expression visualisation and integration platform (expVIP)
Philippa Borrill: The John Innes Centre
Poster 02

- Functional investigation of the N-terminal Acetylation branch of the N-end rule pathway of protein degradation in Arabidopsis thaliana
Mark Bailey: University of Birmingham
Poster 05

- Understanding How Gibberellin Relates to Cell Division Control
Camille Blakebrough-Fairbairn: Cardiff University
Poster 08

- Redox control of the hormonal cross talk in plant immunity
Lucas Frungillo: University of Edinburgh
Poster 14

- A novel method measuring cGMP levels (FlincG) in plants revealed a relationship between cGMP and phytohoromones.
Jean-Charles Isner: University of Bristol
Poster 18

- Waterproof plants: the link between wax biosynthesis and stomatal development
Sarah Jose: University of Bristol
Poster 20

- An edge in combating diabetes with pearl millet.
Jason Kam: Aberystwyth University
Poster 21

- Editing nuclear-encoded RuBisCO in Arabidopsis thaliana with CRISPR/Cas9
Panupon Khumsupan: University of Edinburgh
Poster 22

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Flash Presentations

- Fluorescent phloem-mobile probes allow in vivo quantification of the effects of environmental factors on phloem transport
Kristen Knox: University of Edinburgh
Poster: 24

- Controlling the wave: a role for auxin in Arabidopsis hypocotyl etiolation
Joanna Landymore: SLCU
Poster 25

- Tobacco transformation pipeline and landing pad development for improvement of leaf carbon metabolism and yield.
Patricia Lopez: University of Essex
Poster 26

- CRISPR is on the move: genome editing from rice to wheat
Damiano Martignago: Rothamstead Research
Poster 29

- A molecular framework for the control of organ patterning links cell division and symmetry transition
Laila Moubayidin: The John Innes Centre
Poster 31

- Boundary formation during root development in Arabidopsis thaliana
Elena Salvi: University of Rome
Poster 39

- Transcription factor networks regulating SAG21: a gene at the interface between stress and senescence.
Swapna Nayakoti: Cardiff University
Poster 43

- Comparative biochemical studies of wheat and its wild relative Brachypodium distachyon upon infection by brown rust pathogen Puccinia recondita.
Aizhan Zhussupova: Al-Farabi Kazakh National University
Poster 46

Araport: a 21st century platform for Arabidopsis data integration

Chris Town

Araport

JCVI, TACC, University of Cambridge, Intermine, CyVerse

Araport (https://www.araport.org) is an initiative supported by the NSF and the BBSRC to provide Arabidopsis and plant scientists direct access to a new generation web-based data platform. Users can browse and analyze a large collection of data integrated through Araport. They can also publish their own data for sharing with the community or building analysis workflows.

The platform consists of three main resources. ThaleMine houses a wide array of Arabidopsis genomic information including the latest gene structures and functional annotation, coexpression, orthologs, interactions, pathways, publications, seed stocks, and phenotypes. Users can browse gene reports, run gene list enrichment analysis, export data tables, save work sessions, and share their work with collaborators.

JBrowse hosts close to 100 data tracks that include data sources accessed in real-time. Examples include the most up-to-date gene structures, RNA-seq expression, T-DNA and TDNA-seq, 1001 Genomes Project, whole-genome alignments, and more. Users can also upload and view their own data (BAM or GFF files) for side-by-side viewing or sharing with collaborators. The Science Apps area provides the infrastructure for users to develop web services to expose their own data and “Apps” to display the data or provide analysis modules, thus serving as building blocks for creating discovery workflows. Currently hosting 20+ apps, the future success of Araport depends critically upon community contributions to this data-sharing infrastructure.

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Session 4: Genomics

Software tools and technologies for exploring germplasm diversity

David Marshall

James Hutton Institute

With the advent of high throughput technologies for sequencing, genotyping and phenotyping, plant biologists are being increasingly faced with the task of dealing with the resulting deluge of data at all stages in the pipeline from data capture through quality control through to analysis comprehension. Indeed even dealing with output from a large and complex analysis may often be problematic. Increasingly we have turned to visualisation tools to help us cope ad make our data and those from other studies publically available in a comprehensible fashion.

Using examples from a range of studies in crop species, I will demonstrate how the growing suite of visualisation tools developed by the informatics team at the James Hutton Institute (ics.hutton.ac.uk/software) can be used to accomplish these tasks. These tools run on conventional desktop computers, are freely available and are widely deployed by plant biologists and plant breeders throughout the world.

CyVerseUK

Katherine Denby[1], Robert Davey[2], Anthony Hall[2], Tony Pridmore[3] and David Wild[4]

1- Department of Biology, University of York
2- The Earlham Institute
3- Computer Vision Laboratory, University of Nottingham
4- Department of Statistics and Zeeman Institute, University of Warwick

CyVerseUK is a BBSRC-funded project to set up a UK based node for CyVerse. CyVerse (funded by NSF in the US) provides a high-performance computing environment and tools for scientists to share and analyse large-scale data effectively. It is available for researchers studying all organisms other than humans, and allows research groups lacking in computational capacity or expertise to access extensive data storage and backup, compute power hosted in a number of globally accessible locations, and structured, integrated analysis applications and workflows. Researchers can work on large datasets using publicly available tools and pipelines provided in a single point-of-access, but geographically distributed, infrastructure.

CyVerseUK has been working to establish a UK node to support the UK biological science community's data storage and analysis requirements, and actively encourage and support reuse of data, applications and resources. CyVerseUK hosts computational tools developed through BBSRC-funded projects, enabling them to be globally and easily accessible. These tools include algorithms for genomics, transcriptomics, systems biology approaches, and image analysis.

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Session 4: Genomics

Computational genomic discovery of plant biosynthetic pathways

Marnix Medema

Wageningen University

The last decade has seen the first major discoveries regarding the genomic basis of plant natural product biosynthetic pathways. Computationally driven strategies are now being developed to identify such pathways, which make use of physical clustering, co-expression, evolutionary co-occurrence and epigenomic co-regulation of the genes involved in producing plant natural products.

In this talk, I will introduce and illustrate new methods that make use of computational identification of plant biosynthetic gene clusters and co-expression analysis of biosynthesis-related genes. These methods pave the way for data-driven natural product discovery using state-of-the-art synthetic biology methods.

Workshop Sessions

Workshop 1: Seminar Room

Usage and Application development within Araport.

Organiser: Chris Town

This hands-on workshop is aimed at users of the web portal and will cover the two main applications - ThaleMine and JBrowse.

In ThaleMine:,
- Content and origins of the various data sets that can be accessed through the gene-info pages.
- Making and using lists, including the tools/widgets that operate on lists.
- Templates (pre-formed queries) and how they can be modified to an individual's needs.
- Query Builder that provides a graphical interface to build, save and share SQL queries.
- App Store to get a feeling for what Apps are and how they can be built.

In JBrowse:
- Review the wide range of tracks available and the sources of the data as well as the ways in which data can be searched and manipulated within the application.
- Demonstrate how to import your own data as tracks into JBrowse and review plans for a more user-friendly mechanism for track sharing that is under development.

Users attending the workshop should bring their **own wireless-ready laptops** and should already have created an **Araport account**.
You may also suggest specific topics or questions ahead of time by writing to araport@jcvi.org with GARNet2016 in the subject line.

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Workshop Sessions

Workshop 2: Lecture Theatre

Introduction to CRISPR-Cas, troubleshooting target design and verification of mutants.

Organisers: Vladimir Nekrasov and Amanda Hopes

1. Introduction on CRISPR/Cas as a tool for genome editing

2. Construct assembly methods

3. Target selection and ways to reduce off-targets

4. Ways to detect mutations

5. Case studies:

    - CRISPR/Cas in model and crop plants

    - CRISPR/Cas in diatoms

Growth angle control in higher plants

Stefan Kepinski

University of Leeds

A fundamental feature of plant architecture is that lateral branches are often maintained at specific angles with respect to gravity, a quantity known as the gravitropic setpoint angle (GSA). While the GSA of the primary axis is typically approximately vertical, the GSA values of lateral roots and shoots are most often non-vertical, a crucial adaptation facilitating the capture of resources both above and below ground. Non-vertical GSAs represent an intriguing problem because their maintenance requires corrective growth both with and against the characteristic positive and negative gravitropic responses observed in roots and shoots respectively. Previously we have shown that non-vertical branch GSAs are sustained by means of an antigravitropic offset mechanism that acts in tension with gravitropic response to generate stable, gravity-dependent angled growth. This antigravitropic growth component is auxin transport-dependent and is modulated via TIR1/AFB-mediated auxin signalling within the gravity-sensing cells of the root and shoot. Here I will describe our work to understand the molecular machinery driving angled growth in lateral branches and the factors that differentiate gravity-sensing cells in organs with non-vertical GSAs from those in organs growing vertically.

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Session 5: Signaling

Signal Integration in Nuclear Microdomains

Eirini Kaiserli

University of Glasgow

Light is a fundamental informational signal that regulates plant development, adaptation and survival. Photomorphogenesis and flowering, two of the most dramatic developmental transitions during the life-cycle of a plant, are triggered by an ensemble of environmental stimuli and signalling networks. The talk will focus on investigating the molecular function of TZP1 (TANDEM ZINC-FINGER PLUS3), a natural variant that acts as a transcriptional regulator integrating light and photoperiodic signalling in nuclear microdomains. TZP is a unique nuclear protein that acts as a scaffold for integrating light, hormone and clock networks to accelerate plant growth and establish seedling development during de-etiolation.

Our new findings show that TZP is localised to nuclear photobodies to induce the expression of the floral inducer genes, FLOWERING LOCUS T (FT) and CONSTANS (CO) via a direct interaction with the red light receptor phytochrome B (phyB) [2]. PhyB is essential for recruiting TZP to nuclear photobodies and to chromatin regions on the promoters of FT and CO. In addition to identifying TZP as a new signal-integrating component of photoperiodic flowering, we signify a transcriptional regulatory role to the enigmatic plant nuclear photobodies [2]. Understanding the molecular function of nuclear bodies as transcriptional hubs of diverse signalling components regulating major developmental transitions in plant and animal systems.

[1]- A zinc knuckle protein that negatively controls morning-specific growth in Arabidopsis thaliana. (2008) 105 (44), 17193–17198. http://doi.org/10.1073/pnas.0807264105

[2]- Integration of Light and Photoperiodic Signaling in Transcriptional Nuclear Foci. (2015) 35(3), 311–321. http://doi.org/10.1016/j.devcel.2015.10.008

Oxygen and nitric oxide signalling through functionally diverse targets of the N-end rule pathway

Daniel Gibbs

University of Birmingham)

Oxygen (O2) and nitric oxide (NO) are gases that function as key developmental and stress-associated signals in plants. Investigating the molecular basis of their perception has the potential to identify new targets for crop improvement. The transcriptional response to O2/NO availability in plants is mediated by controlled degradation of plant-specific ERFVII transcription factors by the N-end rule pathway of proteolysis, an ancient division of the ubiquitin proteasome system that marks proteins for destruction based on the nature of their N-terminus.

In the presence of O2/NO, ERVIIs are targeted for degradation via their distinctive Met-Cys- (MC-) initiating N-terminus, whereas reduced availability of either gas permits their accumulation and subsequent activation of downstream genes. We have recently identified the polycomb group protein VERNALIZATION2 (VRN2) as a novel MC-initiating N-end rule substrate, and have linked its regulation by this pathway to several known and novel functions. VRN2 is a core component of the polycomb repressive complex 2 (PRC2), a highly conserved protein complex that regulates the epigenetic silencing of genes by catalysing the deposition of the H3K27me3 mark on chromatin.

We hypothesise that VRN2 acts as a plant-specific link between O2/NO and chromatin dynamics, and that plants control rapid transcriptional changes and longer-term epigenetic responses to the availability of these gases through targeting functionally different proteins to the same degradation pathway.

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Session 5: Signaling

The endodermal barrier - walking the thin line between uptake and protection.

Niko Geldner

University of Lausanne

The supracellular Casparian strip network the endodermis is a highly conserved feature of plant roots, thought to have evolved for balancing the need of a protective seal with that of continued uptake from and perception of the environment. How this intricate structure is built and how the plant manages to ensure a tight, tissue-spanning seal is unknown. In a forward genetic screen aimed at identifying factors involved in the formation of the endodermal diffusion barrier, we identified three mutants that displayed a similar, specific phenotype, consisting of a fragmented, but normally localised Casparian strip. While SGN3 and SGN1were found to encode an LRR receptor-like kinase and a receptor-like cytoplasmic kinase, respectively, SGN2 turned out to be identical to TPST, an enzyme reponsible for sulfating peptide ligands. We therefore speculated that the SGN3-ligand might be a sulfated peptide, yet were able to exclude involvement of previously known ones.

Here I report on the identification of a stele expressed peptide that binds the SGN3 receptor ectodomain and complements the sgn2/tpst CS phenotype at nanomolar concentrations. Moreover, excess ligand leads to strong delocalisation of Casparian Strip domain proteins (CASPs), as well as to massive overlignifiation, specifically of the endodermis - effects that are entirely dependent on the presence of SGN3. I will propose a tentative model as to the role of this putative SGN3-ligand in ensuring the formation of a tightly sealed Casparian strip network.

Poster Abstract 01

A beginner's guide to wheat – www.wheat-training.com

Nikolai M. Adamski, Philippa Borrill, Oluwaseyi Shorinola, Clemence Marchal, Jemima Brinton, Sophie Harrington, James Connorton, James R. Simmonds, Abdul Kader Alabdullah, Karunesh Kumar, Peter Scott, Cristobal Uauy

The John Innes Centre

A large portion of plant biology is focused on model organisms such as Arabidopsis thaliana due to their ease of use relative to crop plants such as wheat. The translation of knowledge between Arabidopsis and wheat is hindered by the fact that learning even simple tasks such as growing and crossing wheat plants requires time and effort while material and methods sections in published articles are often short and cannot substitute teaching aids. This is even more true for more complex topics such as the genomics aspect of wheat.

We have developed the www.wheat-training.com website that aims to help both budding wheat scientists as well as researchers looking to expand their work into wheat. The website is separated into three major sections.

The first section covers the basics of plant growth and crossing with ample detail and images. It also contains efficient protocols for simple wet lab tasks such as DNA extraction. The second part focuses on the available genomic resources in wheat and covers the available genome assemblies, gene models as well as expression and variation data.
The last section is dedicated to the in silico TILLING resource for wheat which can be used to identify mutations in genes of interest. This section also covers creation of genome-specific markers and a guide to identifying the correct orthologue of an Arabidopsis gene in wheat.

The entire website is indexed and can be searched for key words. Relevant passages are intended to be printed off to carry to the lab, glasshouse or field for reference. Additional information such as links to other important websites (e.g. CerealsDB) or online training guides is also available. We plan to expand the content further based on community feedback.

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Poster Abstract 02

Gene expression patterns unlocked by a species-agnostic expression visualisation and integration platform (expVIP)

Philippa Borrill[1], Ricardo Ramirez-Gonzalez[1,2] and Cristobal Uauy[1]

1- The John Innes Centre
2- The Earlham Institute

The majority of RNA-seq expression studies in plants remain underutilised and inaccessible due to the use of disparate transcriptome references and the lack of skills and resources to analyse and visualise this data. We have developed expVIP, an expression Visualisation and Integration Platform, which allows easy analysis of RNA-seq data combined with an intuitive and interactive interface applicable to any species. Users can analyse public and user-specified datasets with minimal bioinformatics knowledge using the expVIP virtual machine. This generates a custom web browser to visualise, sort and filter the RNA-seq data and provides outputs for differential gene expression analysis.

To exemplify expVIP's use we have developed a flexible wheat expression browser comprising > 400 RNA-seq samples (www.wheat-expression.com). This resource provides a comprehensive expression browser for wheat for the first time. We will demonstrate expVIP's suitability for polyploid crops and explore different use scenarios: visualisation of single genes across studies, comparisons of homoeologue expression patterns and the use of heatmaps to identify genes of interest within QTL regions (Borrill et al. 2016). We will also outline how the open-access expVIP platform facilitates the analysis of gene expression data from a wide variety of species by enabling the easy integration, visualisation and comparison of RNA-seq data across experiments.

Poster Abstract 03

Development of an in silico functional genomics resource in polyploid wheat through exome-capture and resequencing of EMS-mutants

Cristobal Uauy[1] Ksenia Krasileva[2,3,4], Hans Vasquez-Gross[2], Tyson Howell[2], Paul Bailey[3], Francine Paraiso[2], Leah Clissold[3], James Simmonds[1], Ricardo Ramirez-Gonzalez[1,3], Xiaodong Wang[2], Philippa Borrill[1], Christine Fosker[3], Sarah Ayling[3], Andy Phillips[5], Jorge Dubcovsky[2,6]

1- John Innes Centre,
2- University of California, Davis
3- The Earlham Institute
4- The Sainsbury Laboratory, Norwich
5- Rothamsted Research
6- Howard Hughes Medical Institute (HHMI), Chevy Chase, MD

Polyploid wheat species originated recently (<500,000 year ago), with most genes being represented by multiple functional copies. These duplicated genes mask the effects of recessive mutations. Gene redundancy, however, also allows wheat to tolerate high densities of induced mutations. We exploited this feature to generate sequenced TILLING populations for tetraploid and hexaploid wheat. We developed an exome capture platform including 82,511 genes and re-sequenced the exomes of 1,535 tetraploid and 1,200 hexaploid wheat EMS mutants. We detected >4 million induced mutations in tetraploid wheat and >6 million mutations in hexaploid wheat. This translates to an average of 21-23 missense alleles per gene and a high probability (>90%) of identifying truncation or deleterious mutation alleles for the majority of wheat genes.

This new resource will accelerate the identification of mutations in the different wheat homoeologs and the generation of plants with complete loss-of function for target genes. We have developed a database and visualisation interface to enable researchers and breeders to access this resource. Seeds of individual mutant lines can be ordered via a direct link from the Germplasm Resources Unit (GRU).

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Poster Abstract 04

Structure and function of Cullin3-RING ubiquitin ligases as novel receptors of immune hormones

Eleanor Adams[1,] Michael J. Skelly[1], Klaus Tietjen[2] and Steven Spoel[1]

1- University of Edinburgh, UK
2- Bayer CropScience AG, Monheim am Rhein, Germany

Eukaryotic gene expression is controlled by regulated degradation of myriad transcription factors and cofactors. Hormone-induced transcriptional reprogramming in plants is especially intimately associated with protein degradation, as many hormone receptors are also E3 ubiquitin ligases. The plant immune hormone salicylic acid (SA) is perceived by members of the NPR protein family that function as SA receptors. NPR proteins contain both BTB and Ankyrin-repeat domains, a typical signature of substrate adaptors for Cullin3-RING ubiquitin ligases (CRL3). Interestingly, SA perception by NPR3 and NPR4 proteins is thought to result in CRL3-mediated degradation of NPR1, suggesting that different substrate adaptors of CRL3 regulate each other's abundance. NPR1 functions as an indispensable master coactivator of SA-responsive plant immune genes. Its degradation is thought to be important for prevention of autoimmunity and paradoxically also for full-scale activation of NPR1 target genes and associated development of immunity.

Despite these findings, it is currently unknown how CRL3NPR adaptors and their substrates interact in vivo. Here we present bimolecular fluorescence complementation evidence in support of the hypothesis that NPR1 is ubiquitinated by CRL3NPR3. Unexpectedly, our data are also consistent with a model in which NPR1 may act as a substrate adapter to target itself for ubiquitination. This potentially novel negative feedback loop may have important structural and functional implications for our understanding of autoubiquitination of eukaryotic CRL adaptors in general.

This work was funded by the BBSRC, Bayer CropScience and The Royal Society.

Poster Abstract 05

Functional investigation of the N-terminal Acetylation branch of the N-end rule pathway of protein degradation in *Arabidopsis thaliana*

Mark Bailey, Adrienne C. Payne, Daniel J. Gibbs

University of Birmingham

Regulating a diverse range of essential processes in eukaryotes, the N-end rule pathway is a highly conserved division of the ubiquitin proteasome system that controls protein stabilities within cells dependent on their N-terminal identity. Recent studies have identified a novel branch of the pathway that specifically degrades N-terminally acetylated proteins - the Ac/N-end rule pathway. In yeast and mammals this pathway plays a key role in regulating protein-complex homeostasis, peptide quality control and signal transduction. At present we do not know if the pathway exists in plants.

Degradation via the Ac/N-end rule involves two key steps: (1) Nt-acetylation by Nt-acetyltransferases (NATs), and (2) recognition and polyubiquitination of Nt-acetylated proteins by one of two specific E3 ligases (Ac/N-recognins) – NOT4 and DOA10/TEB4. We have identified putative homologues of these enzymatic components in the Arabidopsis thaliana genome. We set out to functionally characterise the plant Ac/N-recognins and identify substrates of the pathway in Arabidopsis. Using a yeast based experimental approach informed by proteomic datasets from Arabidopsis, we have identified putative plant substrates and demonstrated conservation in functionality of the Arabidopsis NOT4 orthologues. Phenotypic and biochemical studies point to a key role for the plant Ac/N-end rule in abscisic acid (ABA) mediated responses, suggesting that this novel proteolytic pathway may coordinate responses to abiotic stresses.

Poster Abstract 06

G-quadruplex-dependant gene expression regulates cell wall biogenesis and root hair development in Arabidopsis.

Collette Baxter, Kumar Valluru M, Parker M, Zoulias N, Sorefan, K

University of Sheffield

Root hairs increase the surface area of roots to allow for efficient nutrient and water absorption. Understanding the mechanisms that control root hair growth could lead to their manipulation to increase the efficiency of water and nutrient uptake in plants and therefore reduce the resources required for crop growth.

Root hair growth requires coordinated expression of cell wall biogenesis genes. We show that cell wall biogenesis genes are strongly downregulated in seedlings treated with the G-quadruplex stabilising drug NMM. G-quadruplexes are RNA and DNA secondary structures that form in guanine-rich sequences. G-quadruplexes are associated with the regulation of transcription, telomere maintenance and DNA replication in mammals and yeast. However, little is known about the role of G-quadruplexes in plants.

We find that NMM severely disrupts root hair growth and blocks auxin-induced root hair growth. We will present data supporting the hypothesis that G-quadruplexes are unwound by helicases to facilitate proper gene expression and root hair growth and morphology.

Poster Abstract 07

Phylogenetic relationships between Tunisian and Indian pearl millet germplasms based on seed starch fractions variation.

Mériam Ben Romdhane[1,2], Sandra Pierre[3], Jason Wing Kam[3], Abdelwahed Ghorbel[1], Nèjia Zoghlami[1], Rattan Yadav[3]

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2- University of Sciences of Tunis (UST)
3- IBERS, Aberystwyth University

I am a Tunisian PhD student conducting a two month-training in IBERS under the supervision of Dr. Rattan Yadav. Durning my PhD thesis, I worked on genetic diversity on the most important cereal crops in Tunisia (Barley, oat and pearl millet). Tunisian barley and oat landraces were genotyped using microsatellites markers and compared to other collections (Lebaneese barley (ICARDA) and European oat (CRA fiorenzuola d'Arda during an intership in Italy)) to identify patterns of population genetic structure of these resources.

In the present work, two starch fractions (rapidly and slowly digestible starch (RDS and SDS)) were used as phenotypic traits on a set of 25 pearl millet landraces (15 from Tunisia and 10 from India) to elucidate the partitioning of phenotypic variation between and amongst these two different accessions geographic pools as well as to investigate phylogenetic relationships between Tunisian and Indian landraces. This investigation could explain whether a putative seed exchange between India and Tunisia occurred during the trades of the "Incense Route".

PCA and dendrogram will be drawn on the basis of the RDS and SDS data to bring information on the phylogenic relationships between the Tunisian and the Indian landraces. ANOVA analysis will be performed to assess the phenotypic variation between and within these two groups. The findings will be investigated further in genetic studies.

Key words: Pearl millet landraces, Tunisia, India, phylogenetic relationships, seed starch phenotypic variation.

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Poster Abstract 08

Understanding How Gibberellin Relates to Cell Division Control

Camille Blakebrough-Fairbairn, Jim Murray

Cardiff University

Cell expansion and proliferation are the key processes that drive plant growth. Gibberellin (GA) is a plant-specific hormone that promotes growth and regulates various developmental processes such as germination, floral induction and seed maturation. It has been well established that GA promotes growth through cell elongation by signalling the destruction of a class of nuclear localised growth repressors known as DELLA proteins.

Although this biochemical pathway has been well defined, little is known about the association of GA with cell proliferation. In Arabidopsis thaliana, there are five DELLA proteins that act as co-transcriptional regulators with overlapping but distinct functions. The two main DELLA proteins associated with negative regulation of the GA signalling pathway is GAI (gibberellin insensitive) and RGA (repressor of ga1-3). GAI contains a specific amino acid motif, LxCxE, which is also present in a variety of cellular and viral proteins that interact with a major component of the plant cell cycle known as the RETINOBLASTOMA RELATED (RBR) protein. This motif is responsible for mediating the binding of these proteins to a corresponding pocket domain contained within the RBR protein. Therefore, a putative binding between GAI and RBR may exist in planta, whilst RGA contains a mutated form of this motif, MxCxE. Here, I report on my findings from biochemical, mutagenic and kinematic analysis to determine whether or not there is a functional difference between GAI and RGA, based on their affinity to bind with RBR.

Co-Immunoprecipitation (Co-IP) experiments revealed a binding between RBR and GAI, but not with RGA. Furthermore, kinematic analysis of root elongation, meristem length and cortical cell number of recessive mutant lines for rga and gai in a GA biosynthetic mutant background demonstrated a difference in their ability to partially rescue a GA deficient mutant phenotype. In demonstrating this difference we can thus further our understanding of how GA relates to cell proliferation and how it acts to regulate the cell cycle.

Poster Abstract 09

Multi-scale integration of environmental information in circadian signalling to chloroplasts

Dora Luz Cano-Ramirez[1], Keara A. Franklin[1], Hiroshi Kudoh[2], Antony N. Dodd[1]

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The coordination of biological processes with daily and seasonal changes in the environment is important for the survival of photosynthetic organisms such as plants. To achieve this, temporal information must be extracted from the external environment and integrated into the biological system for correct co-ordination of gene expression. We recently identified in Arabidopsis a signalling pathway that communicates temporal information from the circadian oscillator to another organelle, the chloroplast. This pathway is also regulated by multiple light signals. This signalling mechanism operates through the action of a nuclear encoded protein called SIGMA FACTOR5 (SIG5). Sigma factors are thought to confer specificity to chloroplast transcription by plastid encoded plastid RNA polymerase (PEP).

I am using this signalling mechanism as an experimental model to study how plants integrate complex environmental signals in naturally fluctuating environments. I am combining information from laboratory (*in vivo* and *in vitro*) and field (in natura) experiments to obtain a comprehensive understanding of how the pathway has been shaped to function under fluctuating natural conditions (e.g. during the day and across seasons). The signalling function of SIG5 represents an interesting system to study in natural conditions because it is the first molecular mechanism identified in plants that communicates circadian timing between organelles, and responds to a variety of abiotic stress such as low temperature and salinity. This suggests that the pathway is of considerable biological importance. My data have revealed seasonal patterns of the regulation of the pathway, and suggest that the main regulatory drivers of the pathway under natural conditions include time of day and temperature.

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Poster Abstract 10

YAF9a is implicated in drought stress responses in *Arabidopsis thaliana*

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Plant growth and crop production can be greatly affected by common environmental stresses. Water supply is the single most limiting factor in many countries and drought stress is one of the main factors affecting plant productivity. Chromatin modification and remodelling factors impinge on different mechanisms of abiotic stress responses in plants. Understanding the function of epigenetic mechanisms in the plant responses to drought stress will lead to major advances in the area of crop productivity and tolerance to abiotic stresses. In this study we investigate the hypothesis that the absence of the chromatin remodelling factor YAF9a leads to a state of pre-acclimation to water shortage, most possibly by affecting the expression of stress-related genes.

Previous research in Arabidopsis thaliana has revealed two possible orthologs of the yeast YAF9 protein, designated as YAF9a and YAF9b. YAF9a is involved in flowering-regulatory network by controlling FLC and FT gene expression, through direct or indirect regulation of H4 acetylation levels on these loci. Furthermore, our data suggest that null mutations of YAF9a affect the sensitivity of A. thaliana plants to mild drought stress. Specifically, independent physiology and development experiments have shown that yaf9a-1 grow with only minor differences under mild drought stress conditions compared to optimum watering. Measurements of rosette area at consecutive developmental points, implicate that the two mutants grow almost the same under three watering conditions.

Additionally, analysis of chlorophyll fluorescence of yaf9a-1 plants, suggest that the latter are physiologically unaffected by water shortage. To further investigate the physiological and biochemical changes responsible, a series of complementary experiments are conducted.

Poster Abstract I1

Re-engineering photosynthesis: editing nuclear-encoded RuBisCO in higher plants with CRISPR/Cas9.

Sophie Donovan, Alistair McCormick

University of Edinburgh

The enzyme RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) catalyses net CO2 assimilation in all photosynthetic organisms. However, RuBisCO is slow and cannot fully discriminate between O2 and CO2. Improving the performance of RuBisCO could significantly increase the productivity of C3 crops, such as rice and wheat. The RuBisCO complex is comprised of a chloroplast-encoded large subunit carrying the catalytic site and nuclear-encoded small subunits (SSUs), which may play an important role in determining the catalytic rates. Understanding the significance of SSU variation on RuBisCO's catalytic efficiency in planta is challenging due to lack of a suitable species for SSU transformation. In higher plants, SSUs are encoded by large rbcS gene families composed of 4-22 isoforms, which has hindered the generation of multiple SSU knockout mutants.

To test the potential of the CRISPR/Cas9 system to induce gene knockouts of several rbcS genes simultaneously, we used transient expression assays in tobacco (Nicotiana tabacum L.) leaves. Here, we report CRISPR/Cas9 mediated gene editing using Cas9-sgRNA constructs designed to target regions of shared homology and disrupt two rbcS isoforms. A large deletion was generated by expressing two promiscuous sgRNAs, facilitating simple screening for mutation events by PCR. Targeting two rbcS isoforms led to a 640 bp deletion in both loci and confirmed Cas9 activity at the sgRNA target sites. We have demonstrated the feasibility of using the CRISPR/Cas9 system to manipulate members of the rbcS family in tobacco, with the future aim of developing this approach to re-engineer the RuBisCO SSU family in higher plants.

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Poster Abstract I2

Control of leaf cellular proliferation, differentiation and growth by light: establishing and distinguishing the roles of hormonal- and sugar-signalling

Sara Farahi Bilooei, Enrique Lopez-Juez, Laszlo Bogre

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Light induces the shoot apical meristem to initiate the production of leaf primordia and eventually leaves, but this process is arrested in the dark. Light energy is itself required for leaf growth.

We have in the past observed that, as judged by gene expression signatures, the arrested meristem and primordia in the dark show a strong response to auxin. We now report that they also show a strong "starvation" gene expression. These signatures are rapidly turned by light into strong cytokinin and strong "feast" gene expression. Both coincide with ribosomal protein gene expression and simultaneous cell proliferation, key components of leaf initiation. The leaf primordia transfer to dark leads to disappearance of mitotic reporter activity but this will reappear in the light.

Our data suggest that the seedling meristem and young leaf primordia may specifically experience carbon starvation in the dark, this being quickly repressed when transferred to the light.

Plants' transfer from low light (LL) to high light (HL) also results in extra proliferation and growth. A leaf grown in HL is composed of several layers of larger cells. Thus both multiple layers, and a larger lamina composed of more cells in two dimensions, occur in HL. From those observations, we propose that energy signalling processes are also central to leaf growth under natural, varying light conditions.
The present study aims to identify the exact location of the observed gene expression responses of Arabidopsis, investigate the signalling pathway of the starvation/feast response of meristematic activity, understand what are the different roles of both control mechanisms, and test their further involvement in the response to different light quantities.

Poster Abstract 13

Improving crop herbicide tolerance using directed mutagenesis

Dhirendra Fartyal[1,2], Aakrati Agarwal[1], M.K. Reddy[1], Lorenz Fuchs[2], Anne Maddison[2], Sue Dalton[2], Keith Edwards[3], Dylan Phillips[2], Huw D. Jones[2]

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2- IBERS, Aberystwyth University
3- Bristol University, UK

To meet the global food requirements of a growing population, we must significantly and sustainably increase our crop harvests. However, the availability of land and water, along with limiting abiotic and biotic factors are major obstacles to achieving this goal. Competition from weeds is one such biotic challenge and responsible for massive losses in global agricultural productivity. Weeds are typically controlled using herbicides combined with other farming practices. However, effective weed control remains challenging in many environments and good selective herbicides are difficult and costly to develop, to apply effectively on-farm and anyway not always available. Following commercial success of crops, possessing the ability to tolerate effective, inexpensive and widely-available broad-spectrum herbicides, researchers at ICGEB, India generated triple herbicide tolerant (HT) Indica rice using four gene cassette (EPSPS, ALS, Bar and igrA).

Native Indica rice genes encoding EPSPS and ALS were mutated with PCR based site-directed mutagenesis to generate a proline to serine change at positions 173 and 171 in the two proteins respectively. The transformed rice plants were found to be resistant to all the relevant herbicides with all genes functioning as expected. Along with PCR directed mutagenesis, we are also investigating the use of CRISPR/Cas9 mediated gene editing to produce HT plants. The Cas9 gene was fused with a nuclear-targeted GFP reporter gene via a linker and transformed into cereal model *Brachypodium distachyon*. These Cas9-expressing 'mother plants' were retransformed with gRNAs either with or without template repair sequences to drive the desired targeted edits in the EPSPS and ALS genes. The plants are in regeneration stage and will be analyzed for the desired gene edit.

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Poster Abstract 14

Redox control of the hormonal cross talk in plant immunity

Lucas Frungillo, Steven Spoel

University of Edinburgh

Plants are continuously exposed to a variety of attackers. By recognising the attacker's strategy, plants trigger a hormonal signalling network that tailors an effective immune response. Key players in this network are the hormones salicylic acid (SA) and jasmonic acid (JA). Whereas SA is central to defence responses against biotrophic pathogens, JA mediates responses to necrotrophic pathogens and herbivorous insects. In Arabidopsis, SA is a potent suppressor of JA downstream signalling and gene expression. However, the molecular mechanisms that underpin hormonal trade-offs in plant immunity remain largely obscure. Activation of plant hormonal signalling pathways is associated with profound cellular redox changes. Particularly the redox active molecule, nitric oxide (NO), has been extensively implicated in hormonal signalling during plant responses to biotic challenge through the thiol-targeted post-translational modification S-nitrosylation.

Thioredoxin (TRX) enzymes compose a large family of thiol oxido-reductases that have been shown to display reductase activity towards S-nitrosothiols (protein-SNO). Oxidized TRXs are recycled by specific NAD(P)H-dependent Thioredoxin Reductases (NTR) or Ferredoxin-dependent Thioredoxin Reductase (FTR). Loss of function mutations of NTRs dramatically increased global protein-SNO to levels higher than observed in trx-h single mutants, suggesting the implication of multiple TRXs in controlling protein-SNO level in plants. Here we investigate the role of the TRX/NTR systems in providing specificity to protein-SNO signalling in SA/JA trade-offs during plant immunity. Following pharmacological hormonal treatment, investigation of temporal transcription dynamics of several TRXs clustered in different subgroups, as well as NTRs, revealed recruitment of specific TRX/NTR systems in early stages of hormonal induction. Further, the comparative expression analysis of selected TRXs in wild-type and (S)NO mutant backgrounds (nox1 and par2-1) indicates a still unrecognized interplay between NO signalling and TRXs on plant immunity. Overall, our data suggest that specific TRX activities are recruited to assist in hormonal signalling and cross talk during development of immunity.

Poster Abstract 15

Deciphering the genetics basis of plant-plant interactions

Gina A. Garzon-Martinez[1], Jay Biernaskie[2], Fiona Corke[1], John H. Doonan[1], Anyela Camargo-Rodriguez[1]

1- IBERS, Aberystwyth University
2- University of Oxford

Plant competition for resources, such as light, water or nutrients, is a complex dynamic process that has implications for the formation and diversity of plant communities and ecosystems as well as impacting on agronomical practise for optimizing yield. The processes that affect plant-plant interactions are poorly understood at both the genotypic and phenotypic/physiological levels. For example, traits involved in reproductive fitness can be differentially affected by intraspecific and interspecific interactions. A decrease in a genotype's fecundity is defined as competition whereas an increase can be defined as co-operation.

Some studies have described the phenotypic outcome of kin and non-kin interactions in Arabidopsis thaliana but, even in this model system, the genetic basis remains poorly understood. The aim of this study is to assess the extent of natural variation in the response of plants to neighbours and thereby identify genomic regions associated with changes in life history traits under competitive/cooperative interactions. High-throughput phenotyping approaches will be used to quantify traits related to growth, survival and reproductive success and undertake a genome wide association study (GWAS) to link the phenotype and genotype. It is expected that the results of this study help us to dissect the genetic basis of adaptation to plant density, which could have an impact in the development of plant breeding strategies to improve crop yield.

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Poster Abstract 16

Biochemical and Molecular Mechanisms Underlying Colour Retention in *Capsicum annuum*.

Alexandra Holden[1], Harriet Berry[1], Daniel Rickett[2], Paul Fraser[1]

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2- Syngenta, Jealott's Hill International Research Centre

The Chilli peppers, *Capsicum annuum*, are globally the most widely grown spice product. Colour retention is a key quality trait for C. annuum and other fruits, vegetables, and some cereals. Carotenoid pigments are responsible for these colour related traits. Post-harvest storage results in the degradation of fruit carotenoid pigments, which affects crop quality. Therefore the colour retention trait is essential for breeders to ensure that the crop is economically valuable.

Carotenoid quantity and composition is directly linked to the red colour phenotype, and subchromoplast sequestration mechanisms of these compounds further contribute to the colour phenotype in C. annuum. Capsanthin, and its esters, are primarily responsible for the red colour of C. annuum, therefore, an understanding of mechanisms associated with carotenoid degradation in C. annuum are required to determine how and why chilli peppers lose their colour over time.
Metabolite profiling will be used to assess variation across a C. annuum population, particularly between high and low colour retention accessions. It is hypothesised that molecular, biochemical and structural factors influence the colour retention phenotype. On a genomic level, QTL analysis will identify regions containing candidate genes involved in colour (carotenoid) loss, and the gene expression profile of these genes will be analysed by qPCR and RNAseq. The carotenoid cleavage dioxygenase enzyme family, in particular, will be assessed to determine their role in colour loss. On a structural and biochemical level, analysis of subchromoplast organelles, such as fibrils, will determine the role of these structures in carotenoid storage, sequestration, and degradation, and how this affects colour retention. Fruit cuticle structure will be studied in order to determine whether this plays a protective role in preventing carotenoid degradation and colour loss. Cellular mechanisms, such as lipid peroxidation, will be studied to determine their effect on carotenoid degradation.
This study will provide a detailed analysis of colour retention mechanisms in C. annuum, which will be instrumental in the breeding of high colour retention varieties, and result in a greater understanding of carotenoid degradation mechanisms in this economically valuable crop.

Poster Abstract 17

Enhancing bioethanol production efficiency using transgenic plants that express microbial lignin-degrading enzymes

Shane Houston

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Cellulose in the plant cell wall is the most abundant organic material in the world, and is used to make bioethanol, a quasi-renewable energy source. Lignin is the second most abundant molecule in the cell wall, providing vital roles including mechanical support, protection of cellulose from microbial degradation and also imparts hydrophobicity to the plant xylem to enhance water flow. However, the remarkable recalcitrance of lignin to biodegradation, with the exception of a handful of microbial species, necessitates lignocellulosic biomass must undergo hydrolytic pre-treatment to make it amenable to the enzymatic fermentation of the resultant simple sugars by microorganisms such as *Saccharomyces cerevisiae*. In vitro pre-treatment of biomass to remove lignin is a costly process, and one that often reduces both the overall yield of fermentable sugars, as well as producing compounds which interfere with the subsequent enzymatic hydrolysis and fermentation processes.

Thus, the physical impedance of lignin to cellulases is a significant obstacle in efficient bioethanol production. The aim of this project therefore is to enhance bioethanol production efficiency using transgenic plants expressing fungal/bacterial lignolytic enzymes. These enzymes will be directed to the plant cell wall under a two-component Cu2+ inducible system. We hope that, by decreasing lignin in planta prior to harvest, there will be increased access of cellulose to industrial cellulolytic enzymes, leading to greater yields of simple sugars to be used in fermentation reactions and therefore enhance the production, and economic viability, of bioethanol from these plants.

Poster Abstract 18

A novel method measuring cGMP levels (FlincG) in plants revealed a relationship between cGMP and phytohoromones

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The cyclic nucleotide cGMP has been shown to play important roles in plant development, as well as in responses to abiotic and biotic stresses. Unravelling the function of cGMP in plants was hampered by laborious and time-consuming methods for measuring changes in the levels of cellular cGMP. Fluorescence-based reporters for the real-time non-invasive monitoring of changes in cellular cGMP have until now only been available for animal experimentation. We adapted the FlincG sensor technology for plants to provide a reliable and quick way of detecting cGMP.
The FlincG cGMP reporter in transient- and stably expressing cells shows a dissociation constant for cGMP of around 200 nM, giving it a dynamic range of around 20-2000 nM.

We used this sensor to study the interaction between phytohormones and cGMP signalling. The impacts of abscisic acid, auxin (IAA), and jasmonic acid on cGMP were apparent for applications of exogenous phytohormone concentrations in the nanomolar range. In contrast, brassinosteroids and cytokinins did not evoke a cGMP signal. Protein phosphorylation was tested as a potential mechanism by which hormone-induced cGMP signals are propagated. A rapid change in the phosphorylation status of 15 proteins was observed, nine of which have already been shown to be modified by these phytohormones. We therefore believe that protein phosphorylation might be a valid target of phytohormone-induced cGMP signalling.

Poster Abstract 19

A Constitutively Active Allele of Phytochrome B Maintains Circadian Robustness in the Absence of Light

Matt Jones

University of Essex

The sensitivity of the circadian system to light allows entrainment of the clock, permitting coordination of plant metabolic function and flowering time across seasons. Light affects the circadian system via both photoreceptors, such as phytochromes and cryptochromes, and sugar production by photosynthesis. In the present study, we introduce a constitutively active version of phytochrome B-Y276H (YHB) into both wild-type and phytochrome null backgrounds of Arabidopsis (Arabidopsis thaliana) to distinguish the effects of photoreceptor signaling on clock function from those of photosynthesis.

We find that the YHB mutation is sufficient to phenocopy red light input into the circadian mechanism and to sustain robust rhythms in steady-state mRNA levels even in plants grown without light or exogenous sugars. The pace of the clock is insensitive to light intensity in YHB plants, indicating that light input to the clock is constitutively activated by this allele. Mutation of YHB so that it is retained in the cytoplasm abrogates its effects on clock function, indicating that nuclear localization of phytochrome is necessary for its clock regulatory activity. Our findings indicate that phytochrome signaling in the nucleus plays a critical role in sustaining robust clock function under red light, even in the absence of photosynthesis or exogenous sources of energy.

Poster Abstract 20

Waterproof plants: the link between wax biosynthesis and stomatal development

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Plants produce a hydrophobic waxy cuticle and stomatal pores that help to prevent excessive water loss, and several studies have revealed certain genes that appear to be involved in both wax biosynthesis and stomatal development. This was first observed in barley, when the flower spike wax mutant cer-g was found to have clustered stomata. Since then, further studies in Arabidopsis have revealed other wax biosynthesis genes with pleiotropic stomatal development effects.

We have continued to investigate this phenomenon in barley and Arabidopsis, and identified a second Cer gene in barley whose mutants have a similar stomatal phenotype to cer-g mutants. GCMS analyses revealed very little difference in their leaf wax compositions compared to WT however, which suggests a more complex and indirect link between wax biosynthesis and stomatal development.

We also examined the stomatal patterning of several mutants along the wax biosynthesis pathway in Arabidopsis to determine whether altering specific groups of aliphatics leads to abnormal stomatal development. While novel roles in stomatal development were found for some genes, the link was not clear in this species either. This ongoing work could have significant implications for food security, because an understanding of how plants co-regulate the production of cuticular wax and stomata may be crucial for developing crops that can thrive in the drier and hotter conditions of the future.

Poster Abstract 21

An Edge in Combating Diabetes with Pearl Millet.

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Diabetes is a highly problematic and increasingly prevalent disease world-wide, resulting in more than 1.5 million deaths every year. Management techniques for prevention of diabetes in high-risk individuals as well as affected individuals are mainly through changes in lifestyle and dietary regulation, such as increased consumption of foods with low glycaemic index (GI). However, information as well as availability of low GI foods, especially in developing countries where prevalence of diabetes is on increase, is lacking.

The cereal crop pearl millet (*Pennisetum glaucum*) is one of the most abundant crops grown in countries such as India, providing a staple food for many poor communities. Compared to other cereal crops such as wheat, pearl millet is claimed to have high nutritional content (e.g. proteins, B-complex vitamins, zinc, magnesium and iron), is gluten free, and has a low GI, making it an outstanding candidate to selectively breed for lower GI for use in diabetes control diets. Using starch phenotypes (i.e. resistant starch {RS}, slowly digestible starch{SDS} and readily digestible starch {RDS}) as proxies for GI, the aim of this study was to assess the claim of pearl millet's superiority in starch composition among other grains such as foxtail millet, finger millet, rice, barley and wheat.

Nine randomly picked genotypes from the aforementioned crop species were analysed for their starch content and composition. In comparison to rice, barley and wheat, pearl millet grains had lower RDS and higher SDS content giving it an edge over these species in these characteristics. Such characteristics are important in controlling blood glucose spike, particularly in diabetics.

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Poster Abstract 22

Editing nuclear-encoded RuBisCO in Arabidopsis thaliana with CRISPR/Cas9

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Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the most abundant enzyme on earth and is responsible for net carbon fixation in all photosynthetic organisms. Despite this important role, the catalytic efficiency of RuBisCO is compromised by its relatively slow turnover rate and inability to completely discern CO2 from O2. This indiscrimination leads to the loss of previously fixed CO2. Improving the performance of RuBisCO would increase the efficiency of photosynthesis and thus increase crop yield. RuBisCO is a multimeric enzyme comprised of a chloroplast-encoded large subunit (LSU) and a family of nuclear-encoded small subunits (SSUs). Although the LSU carries that active sites of RuBisCO, multiple lines of evidence indicate that SSUs can influence the catalytic properties of the enzyme.

In *Arabidopsis thaliana*, the SSU family consists of four genes, three of which, RbcS1B, RbcS2B and RbcS3B, are arranged in tandem within an 8 kb region. Due to their close proximity, it has not yet been possible to generate multiple knockouts of these SSU isoforms. In this study, we designed three CRISPR/Cas9 constructs carrying pairs of single guide RNAs (sgRNAs) to target shared homologous regions within the three genes. Stable expression of the constructs in A. thaliana led to large deletions of 6.8 kb within the 8 kb region, as detected by PCR and sequencing. The deletion detection rates for the three constructs were 15%, 12% and 16%, respectively. Current efforts are focused identifying heritable deletions and developing A. thaliana as a platform for examining the contribution of SSUs to RuBisCO catalysis.

Poster Abstract 23

Nucleoredoxin 1 Guards against Oxidative Stress by Protecting Antioxidant Enzymes

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Cellular accumulation of reactive oxygen species (ROS) is associated with a wide range of developmental and stress responses. While cells have evolved to utilise ROS as signalling molecules, their chemically reactive nature also poses a threat. Antioxidant systems are required to detoxify ROS and prevent cellular damage, but little is known about how these systems manage to function in hostile, ROS-rich environments.

Here we show that during oxidative stress in plant cells, the pathogen-inducible oxidoreductase, Nucleoredoxin 1 (NRX1), targets enzymes of major hydrogen peroxide (H2O2) scavenging pathways, including catalases. Mutant nrx1 plants displayed reduced catalase activity and were hypersensitive to oxidative stress. Remarkably, catalase was maintained in a reduced state by substrate-interaction with NRX1, a process necessary for its H2O2 scavenging activity. These data unexpectedly indicate that H2O2 scavenging enzymes experience oxidative distress in the ROS-rich environments they function in and require reductive protection from NRX1 for optimal activity.

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Poster Abstract 24

Fluorescent phloem-mobile probes allow in vivo quantification of the effects of environmental factors on phloem transport.

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The phloem of higher plants translocates not only photosynthetic assimilates but a wide-range of solutes, proteins and RNAs, from source organs to sink tissues. It performs key roles in solute delivery, and also in signalling in a wide-range of systemic and localised processes. The study of the phloem has been hampered by both the delicate nature of the phloem tissues and the location deep within the plants. Phloem transport has traditionally been investigated using radiolabelled carbon sources. However, these assays lack resolution, flexibility and are difficult to perform in vivo in smaller species.

We have previously described a range of phloem-mobile fluorescent probes and here we show their novel application to studies measuring the effects of environmental and developmental factors including light, time, temperature and age on the rate of phloem transport in Arabidopsis seedlings. The use of these probes as new imaging tools, give important insights into the regulation of phloem transport.

Poster Abstract 25

Controlling the wave: a role for auxin in Arabidopsis hypocotyl etiolation

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Hypocotyl elongation upon germination is one of the first growth events in a plant's life. Rapid, anisotropic, elongation of this organ is necessary to push the shoot apex out of the soil and into the light. Hypocotyl organ elongation is the result of a cellular level, acropetal, wave of cell expansion.

Our aim is to understand what controls this wave of elongation and thus the rapid growth of the hypocotyl. The phytohormone auxin is one obvious candidate given its role in regulating growth; in Arabidopsis, it is unclear whether auxin contributes to etiolated growth: one report concludes there is no role for auxin transport; however, in the auxin over-producing mutant superroot hypocotyl elongation is reduced3. In etiolated pea and lupin seedlings, there is a large body of work indicating that auxin represses elongation and that basipetal auxin transport and responses regulate this process.

Our aim is to pin down the role of auxin transport in etiolated growth of Arabidopsis seedlings, with a focus on the onset of rapid growth (24h post-germination) and the start of the elongation wave.

In order to determine where auxin is made and sensed, we investigated the patterns of auxin synthesis (YUC2- and YUC4::GUS) and response (DR5- and GH3::GUS); auxin synthesis and response are excluded for basal hypoctyol cells where the elongation wave begins. Applications of auxin (2,4-D, IAA, and NAA) and an auxin transport inhibitor, NPA, both delayed the onset of rapid growth and slowed the rate of growth once it began. We have begun characterising the auxin transport network at the start of the elongation wave, focusing on the PIN and AUX/LAX families of auxin transporters. Our data indicates that basipetal auxin transport is involved in regulating the elongation wave in Arabidopsis. Our working hypothesis is as follows: high levels of auxin or its response inhibit growth. Once the auxin is cleared out of cells they are able to elongate more quickly, thus pushing the hypocotyl out into the light.

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Poster Abstract 26

Tobacco transformation pipeline and landing pad development for improvement of leaf carbon metabolism and yield

Patricia Lopez

University of Essex

A new "green revolution" is needed in world agriculture as it has been estimated that increases of 50% in the yield of grain required if food supply is to meet the demands of the increasing world population. Improved photosynthesis has been identified as a potential target to increasing crop yields.

Our project aims to improve leaf photosynthetic carbon metabolism by overcoming points of limitation in the overall process, primarily by overexpression of a number of native and foreign genes. To pursue this, we have established a tobacco transformation pipeline which includes the design and building of constructs, plant transformation, screening and selection of T0 and T1 transformants and T2 analysis.

For construct building, we are currently using Golden Gate cloning, which has allowed us to efficiently build single and multi-gene constructs. We consistently achieve a great number of independent lines per construct with a desirable range of transgene expression at both RNA and protein levels; furthermore, some of the manipulations have yielded plants with the desired increased photosynthetic rates and biomass. Additionally, we are working on the development of tobacco lines carrying a CRISPR/Cas9 based "landing pad system" which will allow us to direct the transgenes into a specific known location on the genome. This poster will present details on our transformation pipeline and progress on the landing pad development.

Poster Abstract 27

Understanding chloroplast development and its regulation with the help of the *cue8* mutant

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Chloroplasts require over 2000 nucleus encoded proteins involved in their biogenesis, function and regulation while their plastome codes only for about 100 proteins. The multi-protein translocon complexes in the outer and inner chloroplast membranes govern the import of those cytosol-synthesised proteins.

In order to understand the regulation of chloroplast biogenesis, an Arabidopsis mutant, *cue8*, showing reduced photosynthesis-associated nuclear gene expression and slow greening phenotype was isolated. CUE8 participates in an early, central, aspect of plastid biogenesis. The defective CUE8 protein correlates with inefficient plastid development throughout the plant, microscopy of seedlings revealing the poor distribution of chloroplasts in mutants.

Chloroplasts of the *cue8* plants are found to have reduced levels of PEP (Plastid Encoded Polymerase)-driven transcripts and yet, paradoxically, elevated NEP (Nucleus Encoded Polymerase)-driven transcripts. The plastome copy number was also found to be higher in *cue8* plants though there was no clear difference in levels of plastid DNA polymerase. Furthermore we have employed forward genetics to deepen our understanding of chloroplast biogenesis by searching for suppressors of the cue8 mutation

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Poster Abstract 28

Investigating Genetic Variations Associated with High Micronutrient Content in Pearl Millet (Pennisetum glaucum) For a Sustainable Future

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Society is demanding sustainable interventions to mitigate the challenges associated with micronutrient malnutrition, and global food insecurity. On a global scale, three billion people suffer from micronutrient malnutrition, with most of them living within poverty stricken areas in developing countries. Within developing countries, iron and zinc deficiencies are the most prevalent nutritional disorders, with severe health limiting consequences. More than 200 million of the world's most nutritionally insecure people are dependent upon pearl millet as their main dietary staple crop; therefore micronutrient biofortification of pearl millet is a promising solution in preventing/combatting micronutrient malnutrition.

Using a germplasm collection consisting of 250 accessions (The Pearl Millet Inbred Germplasm Association Panel, PMiGAP); we aim to identify candidate genes involved in micronutrient accumulation. This will facilitate the development of micronutrient dense varieties. The PMiGAP has been genotyped using GBS in order to identify genome wide SNPs that segregate across the panel. The PMiGAP entries have also been extensively phenotyped for micronutrient content by ICP-AES and anti-nutrient compounds such as phytate. Other anti-nutrient compounds such as Apigenin and Luteolin have also been identified using HPLC-MS. This process of genotyping and phenotyping will facilitate the identification of parts of the pearl millet genome segregating with micronutrient content.

Once such genomic regions are identified, they will be aligned with the whole genome sequence of pearl millet in order to identify candidate genes underlying the variations of micronutrient content. Findings from this study will help to improve the micronutrient content not only of pearl millet, but also of related species using the power of comparative genomics.

Poster Abstract 29

CRISPR is on the move: genome editing from rice to wheat

Damiano Martignago

Rothamstead Research

From the very beginning of the CRISPR/Cas9 revolution, plant scientists were quick to adapt this technology to plants in different crops and model species and to try to prove that the modifications obtained with this system were heritable. Cas9 enzymes specifically optimized for plants were designed for dicots and monocots and since then the number of reports of modifications in different species has grown rapidly. In the lab of Prof. Fabio Fornara, Department of Biosciences, University of Milan rice adaptation to Europe was studied. With the availability of mutant lines being extremely limited, the emergence of genome editing technologies allowed the use of CRISPR/Cas9 to target genes of interest, in particular the two main rice florigens, Hd3a and RFT1. These two genes lie adjacent on the genome which means obtaining a double mutant by crossing single mutants from TILLING populations is virtually impossible. However, using CRISPR/Cas9 technology, mutant lines for the individual genes were successfully obtained.

Success in rice has not been equalled in wheat and there is currently only one paper published that reports CRISPR/Cas9 application. At Rothamsted Research, the problem of a very low efficiency of the Cas9 enzyme in wheat is being tackled using a combinative approach. First, we are producing wheat lines that constitutively express a wheat-optimised Cas9, in order to reduce the variability of de novo introduction and expression of Cas9 during each transformation procedure. We are also using optimised Cas9 enzymes to alter the sequence of a number of target genes with easily recognisable phenotypes which will assist with demonstrating the success of the technique. Results of the successful rice CRISPR/Cas9 editing of florigen genes will be presented plus progress with transfer of the technology to wheat.

Poster Abstract 30

Phytohormone regulation of nitrate-mediated lateral root development

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The John Innes Centre

Nitrogen, in the form of nitrate, is an essential nutrient for plant growth and productivity. Spatial and temporal availability of nitrate is known to regulate lateral root development along the primary root axis; but it is unclear how root cells are able to activate different developmental programs depending on prior nitrate exposure. We are using a combination of laboratory experiments and mathematical modelling to explore the role of phytohormone modulation in this decision-making process and in nitrate-mediated lateral root development generally.

Experiments on auxin reporter lines, DR5::VENUS and DII::VENUS, and the cytokinin reporter line, TCS::GFP, have revealed that low nitrate levels increase auxin availability in the vasculature, whilst high nitrate levels increase cytokinin response. These findings have been incorporated in our in silico model that uses auxin and cytokinin concentrations within discrete root cross-sections (metamers) to determine entry into, and progression through, lateral root development. Our initial results suggest that the auxin-to-cytokinin ratio is critical for modulating cell fate decisions that determine priming, initiation and elongation developmental programs. Studies on specific regulatory components of the pathways are currently underway to further elucidate the mechanisms that control lateral root development.

Poster Abstract 31

A molecular framework for the control of organ patterning links cell division and symmetry transition

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Plant post-embryonic growth involves the formation of almost all organs of the individual where cells actively divide, tissues are specified and organs are shaped to fit their purpose.

The female reproductive organ in angiosperms, the gynoecium, provides a unique system to study processes of tissue specification and polarity, because it is patterned very precisely into individual tissues with different polarities.

At the distal end, the gynoecium is topped with a radial structure called the style, which ensures efficient fertilization. In the model plant Arabidopsis thaliana, we have shown that the style is formed as the gynoecium is undergoing a transition from bilateral to radial symmetry in the apical region. This event is controlled by two bHLH transcription factors, SPATULA (SPT) and INDEHISCENT (IND). SPT and IND are necessary and sufficient to impose radialization at the gynoecium apex and they do so by regulating the distribution of the phytohormone auxin. Loss-of-function ind spt double mutants fail to acquire radial symmetry in the apical region giving rise to split styles. Incoherent orientation of cell-division plane and a final stop in cell division are observed where the central clefts of the split styles arise.
Since cell division without patterning produces disorganised tissues, we are testing the hypothesis that cell division and symmetry transition during style development are coupled and coordinated.

Here we show that SPT and IND, besides controlling the auxin flux, exert control of the core components of the cell-cycle machinery, specifically A-type and D-type cyclins. By means of genetic and molecular experiments we show that specific cyclins are expressed in the style region and transcriptionally controlled by SPT and IND. Furthermore, a role for cyclins in radial symmetry formation is revealed in vivo, highlighting the importance of the cell-cycle regulation in radial symmetry establishment.

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Poster Abstract 32

The CDKG1 protein kinase is essential for male meiosis at high ambient temperature

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The Arabidopsis CDKG gene defines a clade of cyclin-dependent protein kinases, structurally and functionally related to kinases found in the Ph1 locus that suppress homeologous recombination in wheat. We recently demonstrated that CDKG1/CYCLINL is essential for synapsis and recombination during male meiosis at high ambient temperature.

In order to determine if the loss of CDKG1 preferentially affects class I or class II crossover (CO) pathways, we crossed cdkg1-/- mutants with plants lacking MLH3 (class I COs) or MUS81 (class II COs). In both cases, we observed an additive effect, suggesting that CDKG1 is involved in both pathways or alternatively at the point where the decision is made to follow a class I or class II CO pathway. In addition, we found that CDKG1 is alternatively spliced in a temperature-dependent manner.

The two splice variants have distinct sub-cellular localizations suggesting a subspecialisation of isoform function as was observed with the CDKG1 mammalian counterpart CDK11.

Poster Abstract 33

Describing the importance of cell cycling regulators and the weak influence of environmental conditions on dynamic chromatin reorganisation in *Arabidopsis thaliana*, utilising 'omic sequencing.

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Cardiff University

Unweaving the effect that chromatin structure has on the expression patterns of eukaryotes has identified the complex role of epigenetic influence on genomic functioning. Additionally, interlaced with the nucleosomal patterning is the mobile and labile protein structures bound to the genome such as Transcription Factors and activation complexes. Combining differential intensities of MNase-seq and total RNAseq in Arabidopsis thaliana has enabled the mapping of small complexes surrounding genomic features and their differing strengths of correlation to gene expression. Significant chromatin reorganisation effects present in response to knock-out/over-expression of the core cell cycling regulators E2Fc and CYCD3, respectively, however in a Light-Dark growth comparison model chromatin reorganisation effects are minimal, suggesting temporal conditions may be insufficient for significant chromatin change.

While the role of these ever fluctuating epigenetic structures are complex, this investigation has demonstrated how they can result in changes to functionally important genes and pathways, which are here described. Furthermore, the effect of differential digestion level demonstrates the apparent suppressing effect that high MNase use has on small particles and loose binding labile protein complexes. Contrasting between digestion of varying strengths highlights the sensitive features that can be missed in an over stringent approach.

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Poster Abstract 34

Molecular mechanisms involved in controlling root meristem cellular differentiation: the Auxin/Cytokinin crosstalk

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Arabidopsis thaliana root meristem growth and maintenance, depends on complex regulatory network involving the activity of cytokinin and auxin hormones. In particular, cytokinin promotes meristem cell differentiation at the transition zone by modulating the auxin distribution along the meristem through the regulation of the auxin efflux carriers PIN-FORMED. The auxin gradient is then interpreted at cellular level by specific AUXIN/IAA-INDUCIBLE (AUX/IAAs) and AUXIN RESPONSE FACTORs (ARFs) which determine gene expression reprogramming and individual cell response toward proper developmental output. The regulation of the availability of AUX/IAAs and ARFs in the meristem is therefore of critical importance to respond properly to auxin stimuli. It has been demonstrated that during the initial growth phase of root meristem development, cytokinin regulates the AUXIN RESPONSE FACTOR19 (ARF19) in order to promote cellular differentiation.

Here we show that cytokinin controls the activity of ARF19 and its paired AUXIN RESPONSE FACTOR7 also in the root meristem maintenance phase. We also demonstrate that ARF19 is required for reaching cytokinin hormone levels necessary for ARABIDOPSIS RESPONSE REGULATOR1 protein activation. These results highlight a strict dependence between cytokinin-mediated cellular differentiation and the activity of several members of auxin signalling pathway. At the transition zone in particular, cytokinin seems to allow precise cellular response to auxin hormone by supporting the expression of distinct ARFs, the very effectors of auxin signal, whose function is necessary to drive cellular differentiation in order to set and maintain the root meristem size.

Poster Abstract 35

Development of rapid and high throughput protocols to extract slowly, rapidly digestible and resistant starch from pearl millet grains.

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The over-consumption of foods with rapidly digestible carbohydrates is one of the factors for the onset of diabetes. Pearl millet is amongst cereals which appear to be beneficial against diabetes, due to its slower starch digestion properties. Starch is a carbohydrate which is digested in two phases corresponding to rapidly (RDS) and slowly (SDS) digestible starch. RDS is responsible for a rapid glucose release leading to increased glycaemic index within 20 minutes after ingestion, whereas SDS allows for a slower release over two hours. Resistant starch (RS), which is not digested, has health benefits including lengthening the period of satiety. Due to rapid rise in numbers of diabetics around the globe, it is getting important to analyse starch digestibility of food grains.

However, the classical biochemistry protocols for starch digestibility analysis by Englyst (1992), which are still followed widely, are time-consuming. Here we report a modified starch digestibility method with improved throughput that is compatible with large-scale analyses of entries, such as Genome Wide Association Studies. We simplified and miniaturized the assays from 800 mg analysed in 50 mL tubes down to 100 mg in 5 mL tubes. This allowed doubling the number of samples passed in one day (36 samples), reducing the number of operators from two to one, and reducing the number of experimental hours, thus allowing for computer analysis to be done in the same day. An example of the type of results obtained on pearl millet seed germplasm using the original and the modified protocols will be shown. With two replicates, we obtain sufficient reproducibility so as to select for genotypes with lower RDS, higher SDS and higher RS.

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Poster Abstract 36

Agriculture-to-nutrition: Improving calcium content of crops through crop genetics to promote bone health

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As world population both grows and ages, morbidity rates due to Calcium (Ca) deficiency disorders, mainly osteoporosis, are increasing tremendously. The World Health Organization proposes osteoporosis as second major global healthcare concern after cardiovascular diseases with treatment costs forecasted to upsurge to $131.5 billion by 2050. Calcium from food sources are more bioavailable and readily absorbed by body than mineral supplements. However, most widely consumed staple crops do not have sufficient concentration of Ca and other micronutrients in their grains. Among all the cereals, finger millet is the richest source of Ca (350mg/100gm edible portions), which is 10-fold higher than brown rice (33mg), wheat (30mg) and maize (26mg).

Using chemical phenotyping, we have characterised a diverse collection of finger millet germplasm for Ca and other micronutrients (iron, zinc, magnesium, sodium, potassium), and have generated 156,157 SNPs by using genotype-by sequencing. These data are being analysed using genome-wide association studies to identify genomic regions associated with accumulation of Ca and other micronutrients in finger millet grains. Genetic factors that enable higher Ca accumulation in finger millet will be compared to those of other cereals. These findings will serve as a basis for not only further improving nutritional aspects of finger millet but also other staple crops. Such crops will be a tremendous boost for smallholder farmers, agriculture sectors, and food industries. Availabilities of such crops will help develop Ca-rich functional foods and their consumption will ultimately benefit public health and reduce economic impacts of osteoporosis.

Keywords: Finger millet, Calcium, bone health, genotyping-by sequencing, Genome-wide association, functional food

Poster Abstract 37

The Development of Tomato Genotypes with Enhanced Xanthophyll Content in Ripe Fruit

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Ripe tomato fruit contain acyclic carotenes, most notably lycopene which is responsible for the characteristic red colour of ripe fruit. The nutritional benefits of carotenoids are well documented. The carotenoids ß-carotene (provitamin A) and lycopene are essential components of the human diet. Recently, the xanthophylls lutein and zeaxanthin have gained attention as oxygenated carotenoids that, when dietary acquired, can reduce the onset of Age-related Macular Degeneration (AMD). This is the fourth most common cause of vision loss globally and the leading cause of blindness in the elderly. The prevalence of AMD is predicted to increase following population ageing.

In the present study, natural variation has been exploited and transgenic genotypes created that contain ripe fruit with varying levels and amounts of xanthophylls. The xanthophylls include zeaxanthin, lutein and violaxanthin. These xanthophylls containing ripe fruit have been created from high ßv-carotene lines introgressed into transgenic varieties expressing different carotenoid hydroxylase enzymes. These lines have been characterised to ascertain the levels and abundance of zeaxanthin present, gene expression within the pathway, the effect on the metabolome as well as sequestration mechanisms that operate to ensure chemically diverse carotenoids can accumulate in chromoplasts.

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Poster Abstract 38

Effects of perturbing expression of the polyamine catabolic AtCuAO gene family on growth and development in *Arabidopsis thaliana*

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Polyamines (PAs) are involved in controlling cell division and elongation, somatic embryogenesis, plant growth, development and senescence. The most common PAs in plants are putrescine (Put), spermidine (Spd) and spermine (Spm). Their concentration in the cell is regulated by a balance between biosynthesis and catabolism. Catabolism occurs through oxidative de-amination of PAs, by the action of amine oxidase enzymes (AOs). In Arabidopsis, copper-containing di-amine oxidases, one of the two classes of AO enzymes in plants, form a gene family of ten members with different patterns of expression. We have taken two approaches to understanding their function.

Firstly, we are examining in detail the effects of insertional mutants in each member of the gene family on levels of PAs and growth and development. We have shown a clear phenotypic response to perturbation of AtCuAO4 (At4g12290) expression. Mutation of this gene resulted in accumulation of Put in leaves before bolting, and increased Spm and Spd after bolting. Plant development, flowering and senescence were delayed in AtCuAO4 mutants. The delay in senescence was related to flowering time as there were no differences between lines in dark-induced senescence. Stalk height was also lower in the mutant lines, and was linked to GA levels.

As a second approach we have transformed wild type Arabidopsis with artificial micro RNAs complementary to multiple AtCuAO family members resulting in a range of developmental phenotypes including changes in leaf development, flowering time, stem morphology and silique production. We are currently analysing these lines, their stability and heritability.

Poster Abstract 39

Boundary formation during root development in *Arabidopsis thaliana*

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Coherent organ growth is ensured by signaling pathways that position boundaries of cell proliferation-to-differentiation transition. Undetermined post-embryonic root growth starts after germination at the root apical meristem (RAM). The mature root is composed of three developmental zones: the meristematic zone, including a stem cell niche, where stem cell daughters transit-amplify; the transition zone (TZ), where cells stop dividing and begin elongating; the elongation/differentiation zone. At the end of embryogenesis, the root is composed only of a stem cell niche and meristematic cells. Therefore, the TZ is established post-embryonically and, during RAM growth, the TZ shifts along the root until the RAM reaches its final size. In this mechanisms, auxin promotes meristematic cell proliferation, prevailing over cell differentiation, which is controlled by cytokinins.

This work aims to unveil the molecular mechanisms that lead to the TZ establishment and, hence, the processes that drive the developmental zonation of the root during its morphogenesis. To define the time-point and the site at which the TZ is detectable, a morphological analysis was performed. Moreover, in the incipient TZ, the expression of the cytokinin-responsive factor ARR12 was found. To clarify ARR12 role in the establishment of the TZ, ChIP-Seq assay was performed and many genes involved in regulating cell proliferation/cell differentiation balance were found. Thus, my data suggest that cytokinins, via ARR12, contribute to establish the TZ at early stages after germination.

Poster Abstract 40

Characterisation of ALPHA CARBONIC ANHYDRASE 7 (ACA7) in Arabidopsis

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Carbonic anhydrases are enzymes that catalyse the interconversion of CO2 and H2O to HCO3- and H+. In Arabidopsis, beta class carbonic anhydrases have been shown to be involved in CO2-induced stomatal aperture regulation and stomatal development; however, nothing is known about the alpha class carbonic anhydrases in plants. The alpha carbonic anhydrase 7 (*aca7*) mutant was isolated using a forward genetic screen based on leaf transpiration. Two allelic knock-out lines of *aca7* have been characterised. Both showed insensitivity to elevated CO2 in their CO2-induced stomatal closure response; however, unlike the beta class carbonic anhydrases, the aca7 mutation did not have any effect on stomatal development, either in terms of stomatal density or stomatal index.

Using purified protein expressed in vitro, we showed that ACA7 has carbonic anhydrase activity. An in silico analysis showed that ACA7 is expressed in the leaves and in the inflorescence. We found that the fusion protein ACA7::YFP is localised to the endoplasmic reticulum at the subcellular level. Additionally, in comparison with the wild type, both aca7 mutant lines had relatively low seed yields when grown under elevated CO2, which may be due to a lower pollen viability. Taken together, these results have revealed novel functions for the alpha carbonic anhydrases of C3 plants, which play a role in CO2 fixation but also in seed production.

Poster Abstract 41

A mitogen activated protein kinase substrate Tandem Zinc Finger Protein TZF9 mediates PTI in Arabidopsis

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Plants recognize conserved microbial features called pathogen associated molecular patterns (PAMPs) via membrane localized pattern recognition receptors (PRRs) and induce immune responses (known as PAMP-triggered immunity, PTI). One of the central components of PTI includes phosphorylation-dependent activation of the three-tiered mitogen activated protein kinase (MAPK) pathway. To date, the known PTI-responsive MAPKs in Arabidopsis thaliana are MAPK3, -4, -6 and -11. One of the MAPK3 and MAPK6 substrates, Tandem Zinc finger protein 9 (TZF9) was shown to be involved in PTI. TZF9 was shown to be localized in cytoplasm and processing bodies (PB). PBs are cytoplasmic mRNA-protein complexes, known to be sites for mRNA processing/degradation/storage or translation arrest. TZF9 showed binding to 18 mer-ribohomopolymers (polyG and polyU) in electrophoretic mobility shift assays (RNA-EMSA).

In another RNA-EMSA with twelve 100-mer probes (named as 1-12 pentaprobe), TZF9 showed affinity towards only one pentaprobe, Pentaprobe 2. Independently, 10 different putative TZF9 target sequences were identified by performing Systemic Evolution of Ligand by Exponential Enrichment (SELEX) assay. These probes showed some sequence similarity with pentaprobe 2. On the basis of these evidences, we hypothesize that TZF9 might target specific mRNA sequences in the PB and thereby, regulate PTI. Additionally, we showed that TZF9 interacts with a calmodulin binding cytoplasmic protein, annotated as a putative RNA ligase. The interaction is independent of MAPK phosphorylation and RNA binding of TZF9 but requires ankyrin domain of TZF9. The putative RNA ligase was also shown to interact with a related protein TZF7, which is a MAPK3 and MAPK6 substrate. Overall, TZFs may link MAPK function(s) to RNA processing in PBs.

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Poster Abstract 42

The polycomb group protein VERNALIZATION2 is an oxygen- and nitric oxide-regulated substrate of the N-end rule pathway of proteolysis

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The N-end rule pathway of proteolysis targets proteins for destruction based on the nature of their N-terminus. In animals and plants this pathway plays a key role in oxygen and nitric oxide (NO) sensing, through controlling the stability of protein substrates that initiate with the residues 'Met-Cys' (MC), which includes the ERFVII transcription factors of Arabidopsis. We have now shown that another 'MC' protein – VERNALIZATION 2 (VRN2) - is also regulated by this pathway in Arabidopsis. VRN2 is one of three plant homologues of the drosophila protein suppressor of zeste 12 (Su(z)12), which functions as part of the polycomb repressive complex 2 (PRC2), a conserved eukaryotic complex that regulates the epigenetic silencing of genes through depositing the H3K27me3 repressive mark to chromatin. One of the best known functions of VRN2 is promoting vernalization, the process by which plants transition from vegetative to reproductive development after exposure to a long period of cold, and it also has several other key developmental functions.

Here we provide in vitro and in vivo evidence that VRN2 is a physiological substrate of the N-end rule pathway of proteolysis. We show that VRN2 is stabilised under hypoxia and NO-limited conditions, that post-translational accumulation of VRN2 during vernalization is mediated by the N-end rule, and that regulation of VRN2 stability contributes to both the hypoxia survival response and regulation of flowering time. We also demonstrate that VRN2 likely evolved from its close homologue EMBRYONIC FLOWER 2 (EMF2), providing new insight into how proteins can become co-opted to the N-end rule pathway during evolution to provide new functions. We suggest VRN2 may provide a link between gaseous signals and chromatin dynamics, and that the N-end rule may therefore coordinate responses to oxygen and NO availability through controlling functionally diverse targets.

Poster Abstract 43

Transcription factor networks regulating SAG21: a gene at the interface between stress and senescence

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SAG21/AtLEA5 is a member of the late embryogenesis associated (LEA) protein family. It is expressed strongly in mature pollen and up-regulated in response to dark, biotic and abiotic stresses in other tissues. Analysis of a 1685 bp upstream region of the SAG21 gene (At4g02380) revealed the presence of cis elements including W- boxes, binding sites for WRKY TFs, MYC motifs, light regulating elements like GATA box and GT1. To investigate the transcription factor (TF) regulatory network interacting with SAG21, yeast-1-hybrid (Y1H) was used to identify WRKY and NAC family TFs that bind to seven overlapping SAG21 promoter fragments. 13 WRKY and 4 NAC TFs were found to bind to the 1685 bp SAG21-promoter.

To establish whether the binding was of functional significance, selected WRKY TFs: WRKY 15, 33, 63 and 67 were transiently co-expressed with a 1685 SAG21p::GUS reporter construct in protoplasts. WRKY15 was found to have no significant effect on SAG21p activity whilst WRKY33 and 67 acted as negative regulators and WRKY63 activated SAG21p activity 3-fold. This indicates that the binding of these TFs has a regulatory effect on SAG21 expression. To further demonstrate these regulatory functions, real-time PCR was used to measure SAG21 expression in mutant (knockdown/knockout) Arabidopsis lines wrky15, wrky63, wrky67 and nac042 when exposed to selected abiotic stresses. The mutant lines showed a reduction in SAG21 expression when exposed to stress compared to the wild type.

To establish the role of the different portions of the 1685 SAG21 promoter in planta, a promoter deletion analysis is being performed by creating a series of constructs with the GUS reporter. These will be transformed into Arabidopsis and the spatial and temporal GUS expression patterns will be analysed in response to a range of biotic and abiotic stresses.

Poster Abstract 44

New regulators of somatic embryogenesis in *Medicago truncatula*

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Somatic embryogenesis (SE) is a specific type of plant reproduction, which is widely used in plant biotechnology. SE is influenced by many factors, including hormones, transcription factors, culture conditions etc.
WUSCHEL-related homeobox (WOX) family transcription factors and PIN auxin transporters are shown to play important and different roles in SE as well as in conventional zygotic embryogenesis. However, involvement of many WOX and PIN genes in SE remains unstudied. The aim of our research is to find new SE regulators among the members of these gene families.

We found that expression of three Medicago truncatula WOX family genes, STENOFOLIA (STF), MtWOX9-1 and MtWOX-11-like, as well as expression of one PIN family gene SMOOTH LEAF MARGIN 1 (SLM1), is associated with SE, according to qPCR experiments. In embryogenic calli, promoters of STF, MtWOX9-1 and SLM1 genes are active in somatic embryos and also in adjacent zones of calli. We also found that overexpression of STF and MtWOX9-1 genes leads to increased embryogenic capacity of calli and correlates with changes in expression levels of several SE-associated genes. Surprisingly, STF loss-of-function mutants also showed increased embryogenic capacity of calli, suggesting that this transcription factor may influence SE in different pathways.

To unravel the mechanisms of these pathways, we are planning to identify targets and interacting partners of discovered SE regulators by using qPCR, EMSA, yeast-two hybrid and BiFC methods.

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Poster Abstract 45

A class of small cysteine-rich pollen coat proteins are key regulators of the hydration checkpoint in *Arabidopsis thaliana* pollen-stigma interaction

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The early stages of post-pollination in angiosperms involve multiple phases of interaction between male and female reproductive tissues. The establishment of the pollen-stigma interaction is proposed to involve a basal compatibility system that enables compatible pollen to be recognised by the receptive stigma. Divergence of components involved in this system could facilitate the establishment of prezygotic breeding barriers that would limit wasted mating opportunities, restrict interspecies gene flow and contribute to reproductive isolation. A diverse family of small secreted cysteine-rich proteins (CRPs) has been identified as having multiple roles in plant reproduction. CRPs found in the pollen coat of members of the Brassicaceae, the pollen coat proteins (PCPs), are emerging as important regulators of the pollen-stigma interaction. One class of PCPs isolated from the pollen coat of Brassica oleracea, the PCP-Bs, have previously been described, but their function was unknown.

In this study, four putative *Arabidopsis thaliana* PCP-B-encoding genes were identified, determined to be gametophytically expressed during the late stages of pollen development and confirmed as pollen coat proteins. Bioassays utilising single and multiple pcp-b gene knockouts revealed that AtPCP-Bs function in the early stages of post-pollination. Pollen morphology was unaffected in pcp-b lines, however mutant pollen grains showed striking defects in pollen hydration, delays in pollen tube emergence, as well as weakened anchoring of pollen grains to the stigma surface. This evidence suggests that AtPCP-Bs, are important components of the basal compatibility system, establishing a molecular dialogue between compatible pollen grains and the stigma.

Ongoing work focuses on analysing molecular evolution of PCP-B genes and identifying stigmatic targets for the AtPCP-Bs. This study sheds new light on the biological and evolutionary significance of CRPs in plant reproductive signalling.

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Poster Abstract 46

Comparative biochemical studies of wheat and its wild relative *Brachypodium distachyon* upon infection by brown rust pathogen *Puccinia recondita*

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An indicator of the national security of any country is the satisfaction of dietary needs of its population. Under current conditions of growing shortage of wheat, humanity might once again face an acute problem of the food crisis. Annual production of wheat on average is about 600 mln tons. It is expected that by 2020 the demand for it may reach more than 840 mln tons. Satisfying this need is a rather difficult task, taking into account the fact that the number of cultivating areas decreases, and wheat yields in most developed countries have already reached the maximum level. Production of high-quality grain in Kazakhstan is an important strategic direction, contributing to stabilization of agriculture, food security of the country and a decent position in the club of grain exporters in the world market. One of the major factors causing significant damage to grain production in Kazakhstan is a brown rust caused by Puccinia recondita, obligatory wheat pathogen common throughout the world, which might lead to a possible loss of yield up to 30-50%.

Model plant *Arabidopsis thaliana* provided unique opportunities for the study of key biological aspects of plant biology, including resistance to disease. However, Puccinia's inability to infect Arabidopsis provided further prospects for Brachypodium distachyon application in rust research. Comparative study of its molecular, genetic and biochemical features with related cereal grains enables us to understand mechanisms of wheat resistance to both abiotic and biotic factors.

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Poster Abstract 47

NBFP orchestrate organ growth and size through tempering endoreduplication in Arabidopsis thaliana

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How plants modulate the growth and definite size of their organs is a fundamental question in plant biology. Typically, organ growth is controlled by coordinated play of cell proliferation, cell expansion and differentiation, however, the underlying mechanisms that set final organ size are still mysterious. Previously, deubiquitinating enzyme UBP14 encoded by DA3 was described to function with the components of anaphase-promoting complex/cyclosome (APC/C) - ubiquitin ligase to modulate endoreduplication and organ growth in Arabidopsis thaliana.

Here, numerous modifiers of *da3-1* were isolated from EMS mutagenized Arabidopsis populations, which influence the ploidy and organ growth phenotypes of da3-1. The *nbfp-1* was found as one of the modifiers that strongly represses the phenotype of *da3-1* at whole plant level but partially at organ and cell ploidy level. NBFP encodes a constituent protein of nuclear bodies that plays a major role in regulation of splicing complex but its role in endoreduplication and organ size control has not previously described. Further studies revealed that NBFP interacts with cell cycle regulators in vivo and in vitro. Thus, our findings proposed a genetic and molecular foundation of NBFP in endoreduplication and plant organ growth regulation.

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Poster Abstract 48

The Arabidopsis LYN1 gene impacts chloroplast development

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Chloroplast development in plants is regulated by light signalling which is perceived by photoreceptors like phytochrome, to drive gene expression in the nucleus through a complex transcriptional network. We have searched for novel genes involved in such a process, and to this effect have carried out mutant screens. The *lyn1* mutant had been identified as a suppressor of the reduced expression of a photosynthesis-related nuclear gene in *hy1*, phytochrome-defective mutant of Arabidopsis. The *hy1* mutation causes a chloroplast defect as mutants cannot perceive light due to failure of photoreceptor chromophore (phytochoromobilin, PØB) biosynthesis.

lyn1 enhances chloroplast density within leaf mesophyll cells by enhancing the chloroplast content in the light. We observe an increased number of plastid genome copies in *lyn1* mutant dark-grown seedlings. Furthermore lyn1 elevates the protochlorophyllide level in etiolated seedlings. All the results suggest that lyn1 rescues the light response defect by increasing plastid development, which allows the accumulation of a small amount of PØB, in turn leading to extra chloroplast development. This eventually results in a clearly increased light response. PØB level measurement supports the hypothesis that lyn1 triggers accumulation of a very small amount of PØB in *lyn1hy1*. Consistent with the enhanced plastid development, more photo-active phytochrome is detectable in *lyn1* than in the wild type.

The LYN1 gene has been identified through a combination of high-resolution mapping and whole-genome, next generation sequencing. The *lyn1* mutation was found on a gene predicted to play a role in the regulation of expression of other genes through a chromatin-based mechanism.

The complementation test which involved crossing hy1 with an insertional mutant in LYN1 (*lyn1*-KO) and *lyn1hy1* with the same *lyn1*-KO confirmed the KO failed to complement the *lyn1* mutation and it also independently suppressed hy1.

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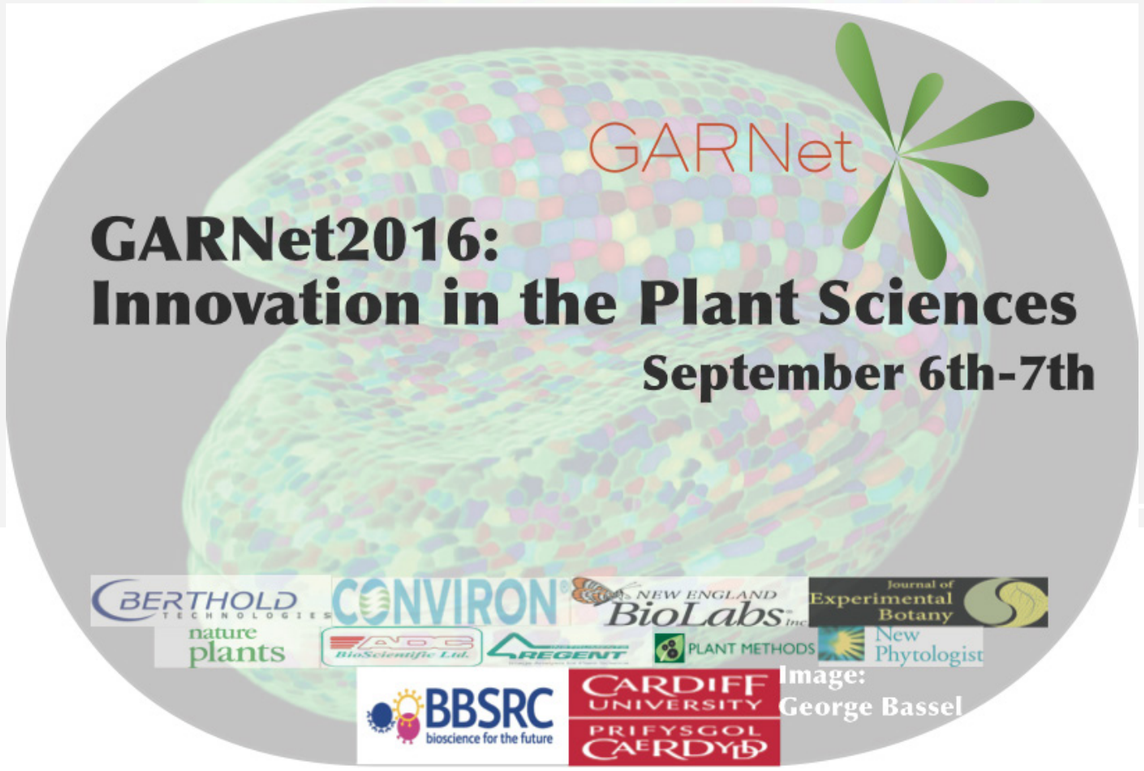

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Natural Variation as a tool for Gene Discovery and Crop Improvement

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  - Detlef Weigel (Tuebingen)
- Ecology and Population Studies
  - Joy Bergelson (Chicago)
- GWAS
  - Magnus Nordberg (GMI)
- NatVar and QTL Analysis
  - Carlos Alonso-Blanco (CNB)
- Genetics of Adaptation
  - Jon Agren (Uppsala)
- Breakthrough Technologies
  - Robin Allaby (Warwick)
- Translational Studies
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