



Gene Editing in Plants



Welcome to the June 2016 Issue of GARNish



Geraint Parry,
GARNet Coordinator

At the time of publication the British public are being asked to make a decision that might have a significant impact on many aspects of scientific research. The current EU funding landscape allows UK researchers to easily participate in pan-European collaborations – a situation that might change, either subtly or more significantly, if there is a vote to leave the EU.

Currently, many plant scientists are frustrated by the pace of the EU decision-making process regarding research and cultivation of genetically modified and/or gene-edited crops. If the UK chooses to 'Brexit' then UKGOV might need to consider these regulations at the individual nation level. Under the current administration this may result in a more permissive regulatory environment. However, this is impossible to predict, especially taking into account the future implications of interacting with other EU countries who have different sets of regulations.

As highlighted in our cover story, no doubt the advent of gene-editing as a viable technology for generating transgene-less plants is an extremely exciting development. Upcoming EU decisions will determine how straightforward it will be for scientists to transfer basic research into the field and whether the full potential of this technology can be realised.

In the UK, the opportunity provided by the Global Challenges Research Fund (GCRF) is an exciting development that will allow plant scientists to take a leading role in the development of tools to benefit those in economically poorer countries. When the initial statements of intent are evaluated by RCUK it will be interesting to understand the level of basic research that will be supported under the GCRF, given the impact that it will have on many Arabidopsis researchers.

Contents

Editorial	2
The GARNet Committee	3
News & Views	4
Engineering with CRISPR-Cas9	8
Plants in Space	10
Funding News	14
SLS16 Meeting Report	20
Brassica Information Resource	23
UKPSF Annual Meeting Report	25
Araport11 Annotation	28
RTD2 Annotation	30
Spotlight on SLCU	32

Special thanks to: Darron Luesse, Lisa Martin, Siobhan Braybrook, Phil Wigge, John Brown, Chris Town, Agnes Chan, Anne-Marie Eckes, Attila Molner, Douglas Pyott, the BBSRC grant holders and plant science researchers at SLCU

The strength of plant science in this country is exemplified by the number of high quality meetings taking place in the UK. This edition of GARNish highlights the recent SLS16 and UKPSF meetings and GARNet are also hosting two exciting meetings later in 2016: 'GARNet2016: Innovation in Plant Science' in Cardiff in September and 'Natural Variation as a tool for Gene Discovery and Crop Improvement' in Cambridge in December. Further details of these can be found on pages 5 and 6.

We are delighted to be able to provide another update from Araport as they continue to develop resources to facilitate Arabidopsis research. In addition we introduce the new RTD2 genome annotation generated by John Brown and colleagues. They are working with Araport in order

The GARNet Committee

David Salt

University of Aberdeen
GARNet Chair Nov 2014–Dec 2016

Jim Murray

University of Cardiff
GARNet PI (from February 2015)

Katherine Denby

University of Warwick
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Antony Dodd

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to make this available as a community resource. This BBSRC-funded research shows that scientific excellence continues to focus on our favourite weed. In the future, hopefully, Araport will be able to develop linkages with similar resources in other plant species, such as the Brassica Information Portal, which is highlighted in this edition of GARNish.

It's unlikely that Arabidopsis will become a staple crop in future Martian colonisation, but it will play an important role in our understanding of what is necessary to grow food in alien conditions. This issue of GARNish includes an article from Darron Luesse that provides an introduction into the excitement and challenges of being involved in space research.

Please enjoy this edition of GARNish, which also details about recent BBSRC grant funding and a Spotlight article on the Sainsbury Laboratory at Cambridge University.

Please follow @GARNetweets on Twitter and Facebook and also remember the '**Weeding the Gems**' blog at <http://blog.garnetcommunity.org.uk>.

Please contact Geraint (geraint@garnetcommunity.org.uk) if you would like to write a guest post.

Views expressed by authors in GARNish are their own opinions and do not necessarily represent the view of GARNet or the BBSRC.



Geraint Parry,
GARNet Coordinator

Following last year's adoption of the UKPSF into the Royal Society of Biology (RSB; see [GARNish24](#)), it has been focusing its work in two main areas. UKPSF recently organised the PlantSci16 conference at the John Innes Centre, from which there is a report on page 28.

In addition, it is supporting the 'Building a Roadmap for UK Plant Science' project, which aims to prepare a comprehensive report examining the directions that the plant science community might take over the next 25 years. This project is being conducted in collaboration with the Institute for Manufacturing from the University of Cambridge. The first part of the process involved connecting with a wide range of stakeholders from across all aspects of UK plant science, including academics, breeders, members of agri-business, environmental consultants, educators and ecologists. These participants were brought together in two-day-long 'roadmapping sessions' held in London and Edinburgh to discuss what should populate the Roadmap.

The GARNet PI and Coordinator attended the London session and found it a useful exercise to discuss the broad range of directions that the Roadmap might take, and to realise the significant effort needed to coalesce these ideas into a coherent document. The Roadmap was also extensively discussed at the PlantSci2016 conference.

Currently, RSB policy fellow Allesandro Allegra is heading up efforts to produce a rough draft of the Roadmap for dissemination to the plant science community. Given the potential importance of this document, it is critical that all members of the community are satisfied with its outcome.

At PlantSci16 the RSB confirmed that the Roadmap will have significant support from the BBSRC, other funding bodies and GOV.UK. This will be critically important for its uptake and acceptance by the wider scientific community and the public.

The most recent UKPSF committee meeting took place at the end of May. It was decided that in the next phase of the Roadmap, UK stakeholders would be asked to help define the most important components of nine categories that have emerged from the Roadmap process to date. Therefore, please look out for your Head of Department (or equivalent), who will be asking your opinion on these topics in the upcoming weeks. Without your input then the Roadmap will not succeed.



Lisa Martin,
GPC Outreach &
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The six months since the last issue of GARNish have passed by in a flash as it's been busy as usual at the Global Plant Council!

We were delighted to recently welcome the Spanish Society of Plant Physiology as our newest Member Society, and following agreement from our Council representatives, we now also accept 'Affiliate Members' (research centres and institutes as opposed to professional societies). The Center for Plant Aging Research at the Institute of Basic Science in Korea, and the Max Planck Institute for Molecular Plant Physiology in Golm, Germany are our first two Affiliate Members, and we look forward to welcoming more. If you would like to find out how your organisation can become a Member Society or Affiliate Member of the GPC, please get in touch!

The GPC has recently itself become a member of the Plant Science Research Network (PSRN), which "will use dedicated workshops to devise novel solutions to broaden participation in plant sciences and to reimagine plant science graduate student and postdoctoral training." It was established thanks to a Research Coordination Network award from the NSF to the Boyce Thompson Institute and the American Society of Plant Biologists (ASPB). With the GPC's involvement we will offer a broader international perspective on this valuable US-based initiative.

Plantae.org, "the digital ecosystem for plant science", which we launched in beta with the ASPB last year, continues to grow as an online hub for plant science news, jobs, policy information, and career, teaching and outreach resources – and much more. The team is still ironing out some bugs and making improvements to the interface so if you haven't already signed up for an account, why not do so today and let us know what you think? It's a great place to host a public or private group, start or join in discussions and network with plant scientists from all over the world! www.plantae.org.

Finally, we were sad to say goodbye to one of our New Media Fellows recently, Amelia Frizell-Armitage – or should I say Dr Amelia Frizell-Armitage! Having been awarded her PhD from the John Innes Centre, Amelia now has a full time position working with a science outreach charity – we wish her every success for the future! We're happy to say that our other New Media Fellow, Bristol University student Sarah Jose, is still with us to manage the GPC's blog (<http://blog.globalplantcouncil.org>) – please get in touch if you'd like to contribute a post!

We've been busy with lots of other things but that's all I have room for, for now! If you'd like to know more about the GPC and what we do, I will be representing the GPC at the Society for Experimental Biology's Annual Main Meeting

in Brighton, 4-7 July. Please stop by the GPC's booth and say hello! In the meantime, don't forget you can interact with us on Facebook (www.facebook.com/GlobalPlantGPC) or Twitter (@GlobalPlantGPC and in Spanish @GPC_EnEspañol), stay up to date with our monthly e-Bulletin newsletter (<http://tinyurl.com/GPCbulletin>), visit our website (www.globalplantcouncil.org) or our blog, and if you have a Plantae account, you can also join our group (<https://community.plantae.org/groups/home/54/>)!



GARNet2016: Innovation in the Plant Sciences.

September 6-7th 2016,
Cardiff University

Registration is now open for the next exciting GARNet2016 meeting. We have put together talks on a broad range of subjects that are linked by their use of novel experimental techniques.

Session 1: Frontiers in Plant Imaging
Session 2: Enabling the Translational Pipeline
Session 3: Plant Synthetic Biology
Session 4: Genomics Tools for Gene Discovery
Session 5: Cell Signaling

In addition we are hosting three workshops that will support new technologies:

> **Pre-Meeting Workshop:** *Finding your Arabidopsis Gene in Wheat.*
Organisers: Philippa Borrill and Cristobal Uauy

> **Workshop 1:** *Usage and Application development within Araport.*
Organisers: Chris Town and Agnes Chan

> **Workshop 2: Introduction to CRISPR-Cas, troubleshooting target design and verification of mutants.**

Organisers: Vladimir Nekrasov and Amanda Hopes

We encourage young researchers to participate in our extended poster session and to apply to give a 2-minute flash presentation.

All information about registration, conference accommodation and the scientific programme can be found at: <http://garnet2016.weebly.com/>.

At time of writing, GARNet has over £500 available to support travel and accommodation for UK-based PhD students to attend the meeting. This money will be distributed on a first-come, first-served basis. Please contact Geraint Parry (geraint@garnetcommunity.org.uk) about the availability of these scholarships.



GARNet Workshop on 'Natural Variation as a Tool for Gene Discovery and Crop Improvement'

December 12-13th 2016,
Cambridge University

The meeting leaders Professor David Salt and Dr Ian Henderson have put together an outstanding selection of world-leading scientists in the area of Natural Variation who will provide presentations in the following scientific sessions:

- > Natural Variation and QTL Analysis
- > Genome-Wide Association Studies (GWAS)
- > Breakthrough Technologies
- > Epigenetic Variation
- > Genetics of Adaptation
- > Ecology and Population
- > Translational Studies

This workshop will take place at Gonville and Caius College, Cambridge University, on December 12-13th 2016. A limited amount of accommodation is still available at the college.

Registration for the meeting and accommodation will open on July 1st. Please see <http://GARNetNatVar2016.weebly.com/> for more information.



Announcement from the UK Plant Phenotyping Network (UKPPN)

Plant phenotyping has recently been selected as a priority research area in the latest round of the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap. The EMPHASIS project (http://www.plant-phenotyping.org/about_emphasis), which was supported by UKPPN, has been selected among only 4 other projects to develop a pan-European research infrastructure for plant phenotyping. Its selection places this research area in a strong position to benefit from the creation of a coordinated European research infrastructure between national partners.

For more details about EMPHASIS please see the presentation at the ESFRI Roadmap kickoff in Amsterdam: http://www.esfri.eu/sites/default/files/articlephotos/Schurr_EMPHASIS_10-3-2016.pdf

In preparation for the UK to become involved as a partner in the EMPHASIS project, UKPPN would like to engage with a broad cross-section of UK researchers in order to:

- > Determine the current status of relevant UK plant phenotyping facilities and expertise available and;
- > Assess which additional facilities and services are required by users in the UK.

This will be used as a basis to create a fully integrated UK plant phenotyping infrastructure, which will then be able to participate in EMPHASIS as a national platform.

In order to make the assessment of available phenotyping infrastructures as efficient as possible, we ask you to please answer the questions in this questionnaire:

https://www.soscisurvey.de/plant_phenotyping/.

This will help us to identify the demands and priorities of the UK phenotyping community.

If you have any questions about this then please make contact with a member of the UKPPN committee (<http://www.ukppn.org.uk/about/>). We would appreciate a rapid reply as we have been asked by EMPHASIS for an overview about UK options and needs.



Agriculture to the Forefront of the Global Challenges Research Fund.

Hopefully most readers will be aware of the extra funding opportunities that have become available with the new Global Challenges Research Fund (GCRF), which provides funding for projects that make a demonstrable difference to Low and Middle Income Countries (LMIC) as rated by the Overseas Development Agency (ODA).

Although this research can be undertaken in the UK, it must be linked to a developmental goal that is relevant to an LMIC country. The GCRF will provide a large pool of £1.5 billion over the next 5 years. In this first call the BBSRC is taking applications for foundational awards valued up to £600K for research over the next 24 months.

One of three focus areas is **Global Agriculture and Food Systems** and should be of significant interest



to plant scientists working in a variety of areas including:

- > Systems that deliver safe and nutritious food for all,
- > Resilience of the food system to climatic variability,
- > Reducing losses throughout the agri-food chain and reusing unavoidable food waste,
- > Agriculture within the context of the wider landscape, and
- > Urban agriculture.

The BBSRC funds will be distributed to UK applicants, but applicants are encouraged to interact with in-LMIC partners to develop the research 'on the ground'. These might be individual institutions or global agencies and research organisations such as the CGIAR centres.

The initial submission deadline for expression of interest proposals is June 22nd so researchers need to rapidly develop their stories. Over the past few years there has been a real-term decline in research funding so the GCRF is a welcome new pool of money.

It will be interesting to learn how projects that lie early on the translation scale will fare during the initial process of triage. We shall see.....

<http://www.bbsrc.ac.uk/funding/filter/gcrf-agriculture-food-systems/>

Turning Gene Loss into our Gain: Engineered Viral Resistance using CRISPR/Cas9 Technology

Attila Molnar and Douglas Pyott,
University of Edinburgh

When we consider improving something – a crop, a computer, or a house – we often look to see what we can add to make it better: a new gene, a bigger hard-drive, or a kitchen extension.

However, sometimes a reductionist approach can be more effective. Sometimes less really is more. In our recent research (Pyott et al., 2016) we used CRISPR/Cas9 technology (the latest and most powerful method for genome editing) to delete the Arabidopsis translation factor eIF(iso)4E, with the aim of generating complete resistance to Turnip Mosaic Virus (TuMV), a major pathogen in field-grown vegetable crops.

This strategy relies on the fact that TuMV specifically requires the eIF(iso)4E isoform to translate its genome, whereas cellular mRNAs can utilise other isoforms to compensate for the loss. By segregating the induced eIF(iso)4E mutations from the CRISPR/Cas9 transgene, we identified transgene-free homozygous mutants that showed complete viral resistance without any adverse effects on the plants' growth. While further work is still required to assess the durability of CRISPR/Cas9-induced TuMV resistance, we believe that a similar approach will be pivotal for generating virus-resistant crops in the near future, especially because many natural sources of Potyvirus resistance rely on loss-of-function mutations in host translation factors.

CRISPR/Cas9 is highly appropriate for translational research (applying what we have learnt in model species to crops) for a number of reasons.

First, the RNA-based guiding of the Cas9 endonuclease in the CRISPR/Cas9 system means that it is both cheap and easy to create Cas9 constructs for engineering crop genomes.

Secondly, the system is more efficient and specific compared to other methods of mutagenesis. Mutagenesis based on chemical (e.g. EMS), physical (e.g. fast neutron bombardment), or biological (e.g. transposon) methods are random in their nature so after the laborious process of screening for the desired mutation, several back-crosses are required to remove off-target mutations.

Finally, CRISPR/Cas9 technology can be used to create non-transgenic genome-edited crops. Even if transgenes are used to deliver the CRISPR/Cas9 construct initially, the induced mutations can be segregated from the inducer transgene in a single generation, resulting in an improved genome differing from the wild type by as little as one nucleotide. Encouragingly, commercial cultivation of CRISPR/Cas9-edited plants and mushrooms have recently been approved by the US government. However, the European Commission is yet to decide on classification and legislation surrounding CRISPR-edited crops.

This is the time for plant scientists to advocate genome editing as an effective and acceptable alternative to classical breeding strategies in order

Wild Type

eIF(iso)4E
mutant



Arabidopsis plants inoculated with a GFP-expressing TuMV clone (TuMV-GFP). UV illumination clearly shows viral infection in wild type plants (top) but not in transgene-free eIF(iso)4E mutants (bottom).

to maintain Europe's competitiveness in the global market. We hope to see this technology enabling plant scientists to apply their research for positive changes to agriculture in the years to come.

Reference: Pyott, D.E., Sheehan, E and Molnar, A. (2016) Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free Arabidopsis plants, *Molecular Plant Pathology*, 2016 Apr 21, doi: 10.1111/mpp.12417

Five things we learnt about CRISPR/Cas9 technology

- 1: The ubiquitin promoter confers strong expression of Cas9 in the germline, which results in a high number of heritable mutations in progenies.
- 2: As in animals, guide RNAs ending with two Gs are very efficient inducers of sequence-specific mutations.
- 3: The T7 endonuclease assay is superior over restriction enzyme-based methods to select lines with the highest level of genome editing in the T1 population.
- 4: Direct sequencing of target genes in the T2 population is the fastest and cheapest way to detect homozygous mutations.
- 5: Growing plants at slightly higher temperatures (25–27°C) can promote flowering and subsequently can reduce generation time. Homozygous, transgene-free mutants can be recovered within 4 months from dipping the the Arabidopsis flowers in Agrobacterium suspension.

Plants in Space: Gravity Experiments with NASA

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The research in our lab involves plants' response to gravity and, to be honest, I've never had many people pay much attention to it. However, when my collaborator Sarah Wyatt from Ohio University and I received funding from NASA to carry out a proteomic analysis of Arabidopsis seedlings grown on the International Space Station (ISS), suddenly everyone became much more interested! The University issued press releases, I was contacted by media science correspondents, even the teachers at my son's school started asking about my project. One of the other parents asked me if I was an astronaut – and most of this happened even before 'The Martian' made it cool to be a plant scientist associated with NASA! This project introduced me to challenges beyond those regularly presented by plant molecular biology, as space research requires overcoming limitations with growth space, equipment, and planetary physics, as well as coordinating with a whole team of NASA-employed scientists, learning NASA acronyms, and relying on a private corporation (SpaceX) for transportation.

One of the major difficulties of sending research into space is adapting standard molecular protocols to the constraints of cargo space, launch timeline, and valuable astronaut time. We were allotted 22 Petri Dish Fixation Units (PDFUs) housed inside four Biological Research



Preparing the BRIC. Photo: Darron Luesse

In Canisters (BRIC) units, meaning we had to figure out how to get enough tissue from 22 tiny petri dishes of 50 mm in diameter to successfully analyse the soluble proteome, the membrane-bound proteome and to provide tissue for an associated RNA-seq experiment. Much of our early work was dedicated to maximizing protein yield from small amounts of tissue, and figuring out how to delay germination so that the plants would not start to grow in the three days between planting and arrival at the ISS.

We planted about 700 seeds per petri dish, growing for only 72 hours, and hoped that any results induced by the stress of crowding would be filtered out in the equally crowded ground controls. Another challenge was getting the seeds to germinate after they reached orbit, four to five days after they were planted. By then, they were already locked into the light-tight BRIC. Based on advice from space research veteran Simon Gilroy, the seeds, sterilized and residing on petri dishes, were given light for 2 hours and then stored cold to prevent germination for at least 96 hours.

Germination could then be initiated by a return to room temperature, allowing activation of our experiment when astronauts removed the BRIC canisters from a transport cooler.

After our methods were decided and the results approved by NASA (a process called "Definition"), we were tentatively assigned space on a launch (SpaceX V, November 2014). It is rather expensive to send things into space, so before research can fly, the team (external researchers and the NASA engineers) perform two dry runs of the experiment, identical to those that will occur in flight except on land, the plants grow in an incubator at the Kennedy Space Centre. These are termed the Science Verification Test (SVT), and the Payload Verification Test (PVT). To move past these benchmarks, we had to hit predetermined goals for protein quantity/quality, RNA quality/quantity, germination frequency and limits on contamination levels.

The timeline for the SVT and PVT were identical to our anticipated launch timeline. On Day 1, we arrived and poured the plates in preparation for planting on Day 2. Day 3 was dedicated to hardware integration. This is the process of handing our materials over to NASA so that the petri dishes can be correctly inserted into the PDFUs and subsequently added to the BRICs. For the real event, the BRICs were loaded onto the SpaceX Dragon capsule on Day 4, while we plated seeds to be used for the ground comparison controls. Day 5 consisted of both the launch and the integration of the ground controls.



Transporting the experiments to Kennedy Space Center. Photo: Darron Luesse

Aside from the excitement of working at NASA, the reality of the biologists' side of preparing Arabidopsis for space flight was rather mundane: we poured plates, sterilized seeds, and scraped them onto plates. In our case, we decided to do most of the preparatory work before travelling to allow for preliminary testing of the seed, sterilisation, and media. Seeds were sterilised in single-plate aliquots, dried on small filter papers, and sealed inside sterile petri dishes for transport. A large batch of growth medium was divided into 500 ml aliquots. This allowed a subset of the seeds and medium to be tested prior to the experiment.

To transport the medium and seeds from St Louis to Cape Canaveral, I lined a hard-sided suitcase with foam seat cushions and wrapped the medium bottles in cushion sleeves and bubble wrap. I was terrified to check this at the airport, because I can't imagine anything looking more like a bomb to an X-ray luggage screener! I left a note explaining the situation, but was still shocked when the suitcase,



All components of the PDFU need to be accounted for!
Photo: Darron Luesse

with medium bottles intact, arrived safe and sound in Cape Canaveral. Once in Florida, our work was performed in a dedicated lab at Kennedy Space Center. Even menial scientific tasks were somehow made awesome, just because they were happening in a lab at NASA!

The process of hardware integration was the first time I had the opportunity to work side by side with NASA scientists. While it seems simple by description, integration is rather complex, because the PDFUs are complicated devices. In advance of the integration, all of the pieces had to be individually cleaned, autoclaved, and catalogued for use.

Integration is performed in a large laminar flow hood as, understandably, NASA is serious about preventing microbial contamination that would likely result in complete experiment failure. A representative from the research team and an engineer tasked with assembling the BRICS, which took about 10 minutes, were the only ones

allowed in the hood. My part as the 'Biologist' was simply to sit in the fumehood and hand over the appropriate petri dish when the technician called "Ready for the Science!"

The dish was then inserted into the PDFU, an air and water-tight seal was added, and assembly was completed. Other members of the team added RNAlater to the injector mechanism of each and began to integrate them into the BRIC. As expected, this process was extremely detailed, down to a defined order of insertion of the screws.

No amount of preparation can mitigate the general difficulties of launching something into space. Weather, equipment, and SpaceX all have to cooperate, making our launch date a moving target. This was easily the most frustrating aspect of space research. It is daunting to consider the number of people and sophisticated technology required to launch something into space on a fireball of liquid oxygen and rocket-grade kerosene – added to the time required so that it can meet and dock with a space station orbiting at about 28,000 km/hr (7.8 km/sec)! Logically, delays are completely understandable but when planning your research and life around them, they can be frustrating! We were originally set to launch on December 3rd, 2014, but the research didn't get off the ground until January 10th, 2015.

During this time we had six official launch dates, and on three of those we were required to fully prep the experiment. After one early cancellation, talk of a December 26th launch had me planning

how to tell my kids Daddy wouldn't be home for Christmas but instead he'd be locked in a sterile lab in the bowels of NASA! The most heartbreaking scrub came with 81 seconds remaining on the countdown, and left me standing on a NASA causeway staring across the water in disappointment that the SpaceX rocket that wasn't going to leave the earth this time. However, when it finally launched, it was clearly the most exciting time in my research career – something I planned for and made was traveling into space.

The SpaceX Dragon capsule docked with the ISS two days after launch, and the following day the astronauts offloaded and stowed the experiment on the ISS. The shift to room temperature initiated germination and the seedlings were allowed to grow for 72 hours. At this time an astronaut used an actuator tool to flood the plates with RNAlater followed by transfer to the MELFI (Minus Eighty Laboratory Freezer for ISS). About a month later, when the Dragon undocked from the ISS and splashed down in the Pacific, the experiment was along for the ride. Upon return to Kennedy Space Center, the BRICS were partially thawed for disassembly and the petri dishes were placed on dry ice for shipment.

I was extremely nervous during the whole month that the experiment was in orbit. There was no way to check progress, no way to know if everything went as designed. Fortunately, germination was great, growth was as expected, and the protein and RNA extractions were completed without anyone accidentally dropping a tube. A feeling that most scientists will understand is the relief I



The team at NASA. (L-R) Sara Wyatt (OU), Proma Basu (OU), Sarah Hutchinson (SIUE), Darron Luesse (SIUE).

felt that I wasn't the guy who messed up a once in a lifetime opportunity to work with NASA!

Ultimately we found a few hundred proteins that were upregulated in the space growth over the ground controls, some being the usual suspects (hypoxia, stress, etc.), but others are less easily explained and open for further research. Now we're back to ordinary bench research, using familiar techniques to make sense of the 'omics results. Although I enjoy it, I also miss the excitement of space research, and I'll definitely submit the next time NASA issues a call for flight proposals. Hopefully I'll get another chance at that once in a lifetime opportunity!

New Arabidopsis Grants

Arabidopsis researchers continue to be very successful in BBSRC responsive mode funding rounds. Here's a round-up of grants awarded to members of our community in the BBSRC Responsive Mode 2015 Round 2. Congratulations to the PIs and the researchers in post working on these exciting projects!

Dissecting a New and Vital Checkpoint in SNARE Recycling and Plant Growth

Mike Blatt,
University of Glasgow

SNARE proteins are central components of a well-defined mechanism for the delivery of vesicles carrying membrane and soluble cargo between compartments within cells and contribute to homeostasis and signaling in all eukaryotes. Cognate (Qa-, Qb-, Qc- and R-)SNARE proteins localise to vesicle and target membranes, and assemble in complexes to drive membrane fusion. So-called Sec1/Munc18 (SM) proteins are known to regulate this process. They form a clothespeg-like structure that 'clamps' and stabilises the SNAREs in complex during vesicle fusion.

Post-fusion disassembly of the SNARE complex is essential to recycle the cognate SNARE proteins and maintain vesicle traffic. Disassembly is achieved by the NSF ATPase, which binds the SNARE complex with the adaptor protein α SNAP. Logic dictates that SM debinding is prerequisite for SNARE complex disassembly, but an understanding of how this process might be regulated is wholly absent. Indeed, most eukaryotes express only one or two α SNAP and NSF proteins, yet maintain vesicle traffic via a large number of different SNARE-mediated

trafficking pathways. Clearly, substantial coordination between trafficking pathways must occur to ensure NSF activity is effectively distributed.

Recent findings from my laboratory showed that manipulating SEC11 binding to the Qa-SNARE SYP121 blocks vesicle traffic via both SYP121- and SYP122-mediated pathways, even though SEC11 does not interact with SYP122. The two Qa-SNAREs share other cognate SNAREs, leading us to observe that the SEC11-SYP121 association is essential, post-fusion, to promote SNARE disassembly and recycle these binding partners. In short, we have uncovered a previously unrecognised checkpoint and a new role for an SM protein in SNARE recycling post-fusion.

Our working hypothesis is that SEC11 debinding is a key checkpoint and serves as a molecular 'clutch' for disassembly of the SYP121 SNARE complex and its coordination with parallel trafficking pathways at the plasma membrane.

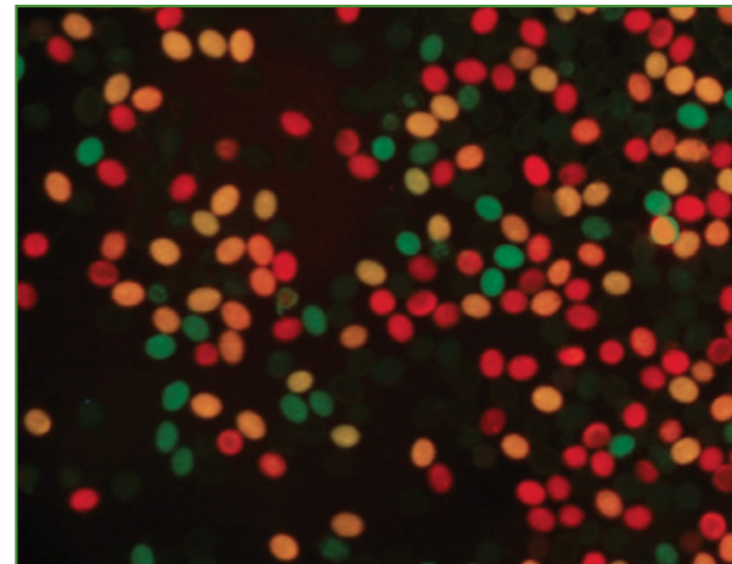
Work is now underway to fully characterise the binding of SEC11 with SYP121 and their association, post-fusion, with α SNAP and NSF in disassembly of the SNARE complex as well as their implications for vesicle traffic, cell expansion and growth. The capacity of plants to survive adverse conditions and reach reproductive maturity critically depends on their ability to continuously adapt to changes in the environment, particularly in response to pathogens. Many studies have identified the control of protein stability as a major regulator of plant responses during invasion and propagation of pathogens, showing that modulation of the stability of key regulatory proteins is required for adaptation to pathogenic infections.

EpiSpiX: Unlocking Plant Genetic Diversity via Epi-modification & Targeted Recombination

Ian Henderson,
GARNet Committee Member
University of Cambridge

Breeding of natural genetic variation remains a vital tool for crop improvement and it relies on the efficiency of meiotic recombination to generate novel segregants. One common limitation is the skewed distribution of meiotic recombination events along the chromosomes, including crop species such as wheat, barley, maize and tomatoes. MeioGenix develops a plant breeding technology (SpiX) that can target and increase the frequency of meiotic recombination in regions of low recombination ("cold regions"). Epigenetic controls seem to have an important role in defining these cold regions and the work my lab has elucidated part of these controls in the model plant *A. thaliana*. This grant will help translating preliminary results into wheat, the most important food crop.

We have seen exciting changes in recombination caused by alterations to plant chromatin. This



Ian Henderson: Screening for meiotic mutants.

grant will allow us to work with MeioGenix and Dr. Pierre Sourdille at INRA in Clermont Ferrand, to translate our knowledge into the complex wheat genome. Specifically, we will modify wheat chromatin in order to control recombination and accelerate breeding.

Pierre Sourdille's lab will provide the expertise and facilities to apply these discoveries in wheat. MeioGenix partners already with Sourdille's lab where the targeted recombination technology SpiX is being applied.

"It is known that the relationship between the genetic and physical distances varies a lot along the chromosomes and between species. This is the consequence of the large variation in the distribution of the recombination initiation events, being genetically and epigenetically controlled. Recent advances that uncover the regulatory role of the chromatin structure are very promising to accelerate the generation of natural variants in plants." says Alain Nicolas, Co-founder and CSO of MeioGenix. *"The timing to call for a breakthrough effort in crops is well suited and the partners of this collaboration are optimal in terms of expertise and complementarity."*

One of the biggest challenges of humankind is feeding a population that is set to reach 9 billion people by 2050. Yields of maize, rice, wheat and soybean all need to increase by 60% and classical breeding might have already attained its limits. In recent years, a series of plant breeding technologies have been developed (e.g. Cas9, Reverse Breeding, SpiX) that can accelerate plant breeding and facilitate the development of improved crops. The technologies developed by MeioGenix and the University of Cambridge will have an impact for plant breeders and contribute to the valorisation of biodiversity.

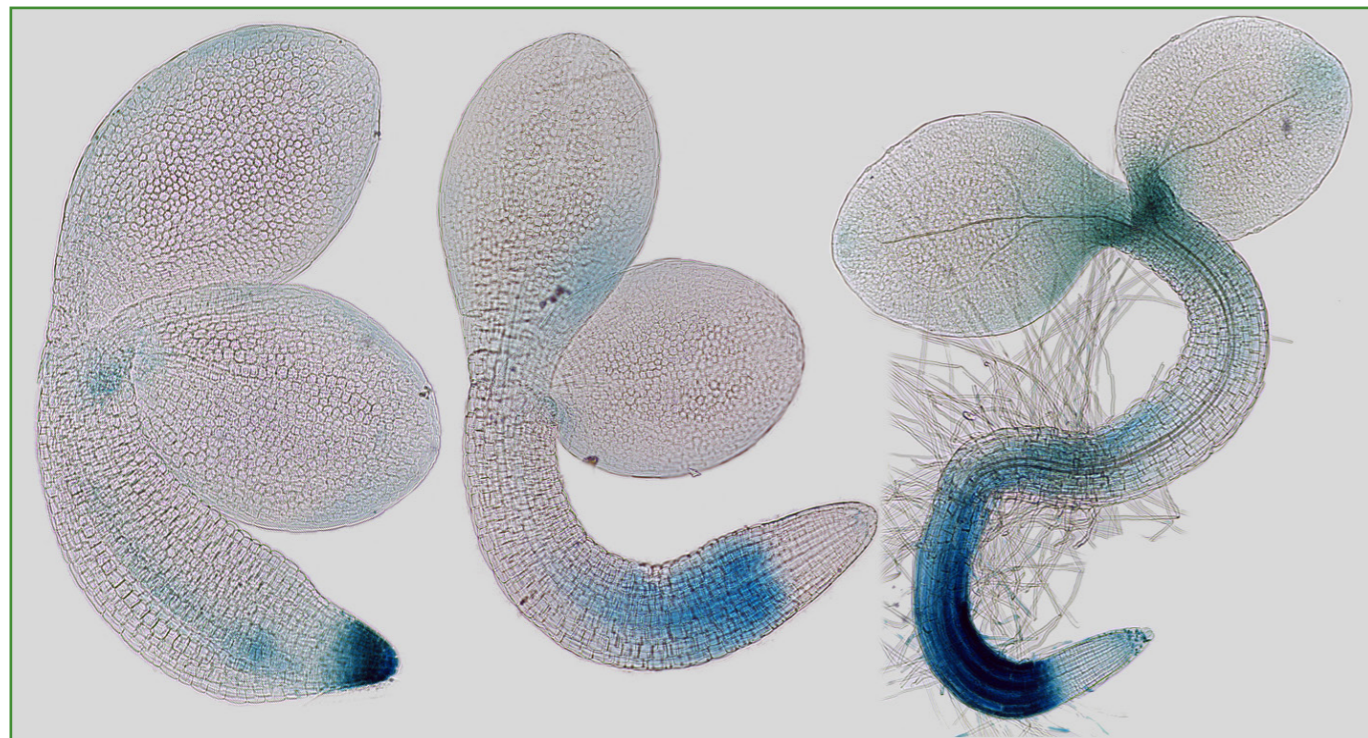
Genetic and Mechanical Approaches to Enhancing Crop Seed Vigour

George Bassel,
University of Birmingham

The majority of agriculture begins with the planting of a seed in the field. Following this, the rapid and uniform establishment of seedlings plays a key role in the future yield and profit for food producers. The “vigour” of seeds describes their ability to establish seedlings across a wide range of environmental conditions, so enhancing this trait remains a key objective of the agricultural industry.

The seed to seedling transition is driven exclusively by cell expansion driven by increases in cellular turgor and modifications to the mechanical properties of the cell wall. Seed vigour may therefore be considered a mechanically driven crop trait, and the ability to unconditionally generate growing force to drive cell expansion.

This BBSRC-funded project will identify



George Bassel: Gene expression dynamics during Arabidopsis seedling establishment.

transcription factors that directly regulate genes encoding the cell wall modifying genes in Arabidopsis seeds. These regulatory factors represent the genetic targets that influence the mechanics of seedling growth, and genetic targets for the modulation of the mechanical properties of the embryo, and therefore seedling vigour.

In collaboration with industrial partner Syngenta, orthologues of these growth-regulating factors from Arabidopsis will be studied using TILLING lines within the crop species *Brassica oleracea*. This can identify novel alleles that enhance seed vigour and crop performance.

In parallel with genetic experiments, cellular level 3D mechanical models of each Arabidopsis and Brassica embryo will be developed.

Computational growth simulations using these cellular templates will enable the cellular basis of organ-level mechanics to be explored, and identify the cellular sites where growth potential is conferred. Understanding the cellular basis of force generation within the embryo will provide targets for the manipulation of this system.

Understanding the Role of the Chloroplast Ubiquitin E3 Ligase SP1 in Abiotic Stress Tolerance in Plants

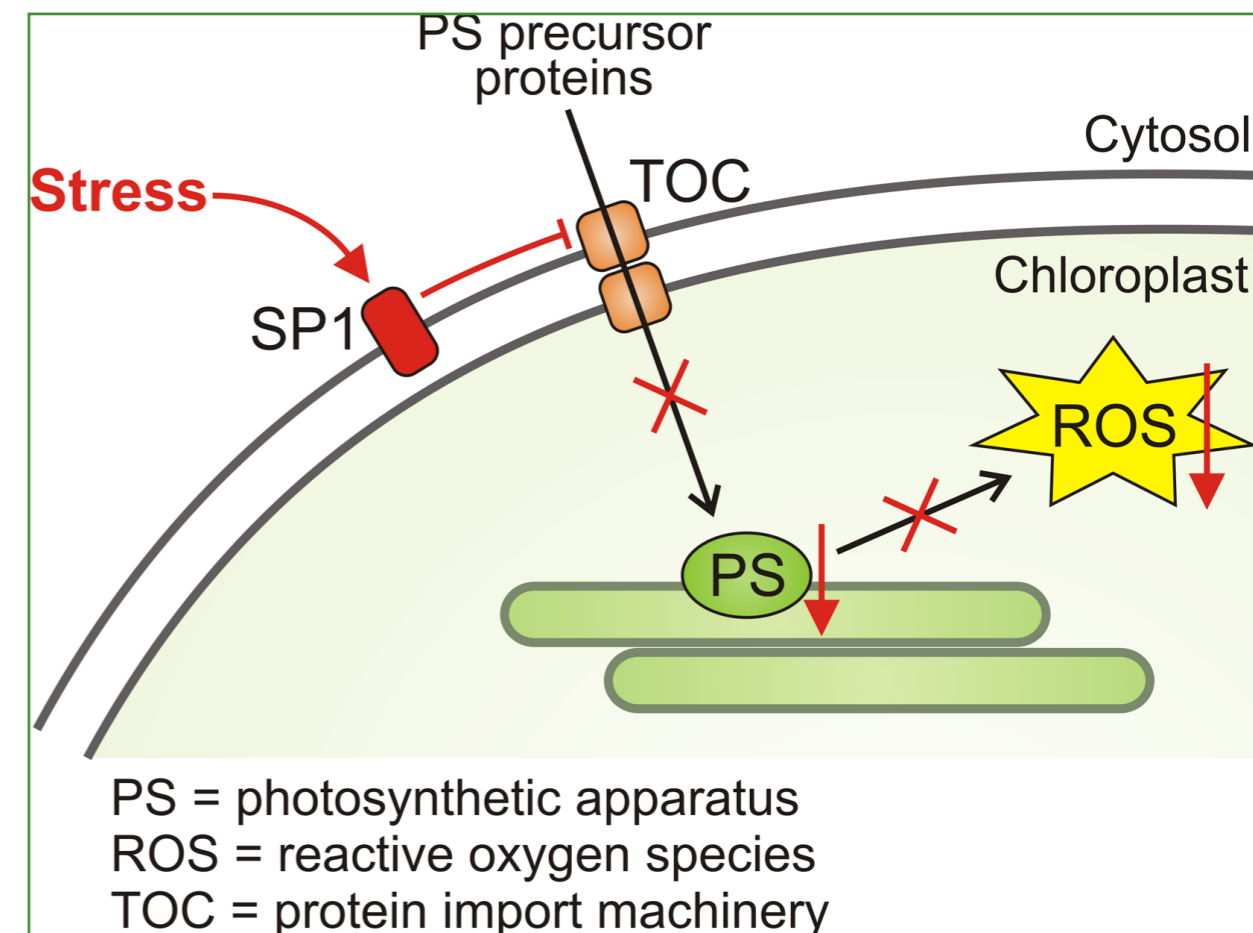
Qihua Ling and Paul Jarvis,
University of Oxford

Chloroplasts are essential organelles in plant cells. They contain the green pigment chlorophyll that, in association with complex multiprotein machinery, plays a central role in photosynthesis – the process that converts sunlight energy and carbon dioxide into sugars. While photosynthesis provides the energetic basis for plant growth, it also generates “reactive oxygen species” (ROS) as by-products. Excessive ROS accumulation can be toxic or even lethal to plants, and this is particularly likely to happen when the environment is challenging. Thus, chloroplasts have a critical role to play in stress responses.

The chloroplast proteome comprises ~3000 different proteins, the vast majority of which are synthesised in the cytosol in precursor form.

As chloroplasts are enclosed by a protein-impermeable double-membrane

system called the envelope, these precursor proteins need to be imported through dedicated protein translocons called the TOC and TIC complexes, located in the outer and inner envelope membranes respectively [1]. Our lab identified an important regulator of the TOC complex called SP1, which is located in the outer envelope membrane [2]. The SP1 protein is a ubiquitin E3 ligase – such proteins are key components of the ubiquitin-proteasome system (UPS), an essential cellular process for selective protein degradation. SP1 mediates the ubiquitination of TOC components, leading to their selective removal by the UPS. Owing to its effect on protein import, such control enables the reorganisation of the chloroplast proteome, which is particularly important during developmental phases when chloroplasts undergo major



Paul Jarvis: Model illustrating the role of SP1 during abiotic stress. Protein import regulation by SP1 under stress conditions delivers attenuated photosynthetic activity as a means to avoid the over-accumulation of harmful ROS. Taken from Ref. 3.

functional changes such as de-etiolation and senescence [2].

Recently, we revealed another critical role of SP1 in stress responses, and this too is closely linked to chloroplast proteome reorganisation [3]. Arabidopsis plants lacking SP1 are hypersensitive to salt, osmotic and oxidative stresses, whereas SP1 overexpressors are more stress tolerant than the wild type. We hypothesised that SP1 acts to deplete the TOC apparatus under stress conditions in order to limit the import of photosynthetic apparatus components, thereby attenuating photosynthetic activity and reducing the potential for ROS over-production and photo-oxidative damage (See figure).

These new findings raise important questions concerning the mechanism of SP1 activation under stress, and the identity of the processes that are controlled by SP1-mediated protein import regulation. Such issues form the basis of this new BBSRC-funded project. We will conduct experiments to elucidate the signalling mechanisms that control SP1's function in stress, and investigate SP1's influence on the chloroplast proteome in stress.

We will also assess the role of SP1 in salt and drought stress tolerance in crops, and anticipate that the project will inform strategies to develop improved varieties that are better able to cope with adverse environments.

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2. Ling, Q., Huang, W., Baldwin, A. and Jarvis, P. (2012) Chloroplast biogenesis is regulated by direct action of the ubiquitin-proteasome system. *Science* 338: 655-659.
3. Ling, Q. and Jarvis, P. (2015) Regulation of chloroplast protein import by the ubiquitin E3 ligase SP1 is important for stress tolerance in plants. *Curr. Biol.* 25: 2527-2534.



Gravitropic setpoint angle control in higher plants

Stefan Kepinski,
University of Leeds

The overall shape of plants, and the space they occupy above and below ground, is largely determined by the number, length, and angle of their branches. Interestingly, the angles at which many root and shoot branches grow out are set and maintained relative to gravity rather than the main root–shoot axis. These angles are known as gravitropic setpoint angles, or GSAs. The GSA values of lateral shoots and roots are most often non-vertical, a crucial adaptation allowing the plant to optimise the capture of resources both above and below ground. Despite the importance of branch angle as a fundamental parameter of plant form, until now, research has been more focused on the mechanisms controlling numbers of lateral roots and shoots, and work on gravitropism has been all but confined to the primary root–shoot axis.

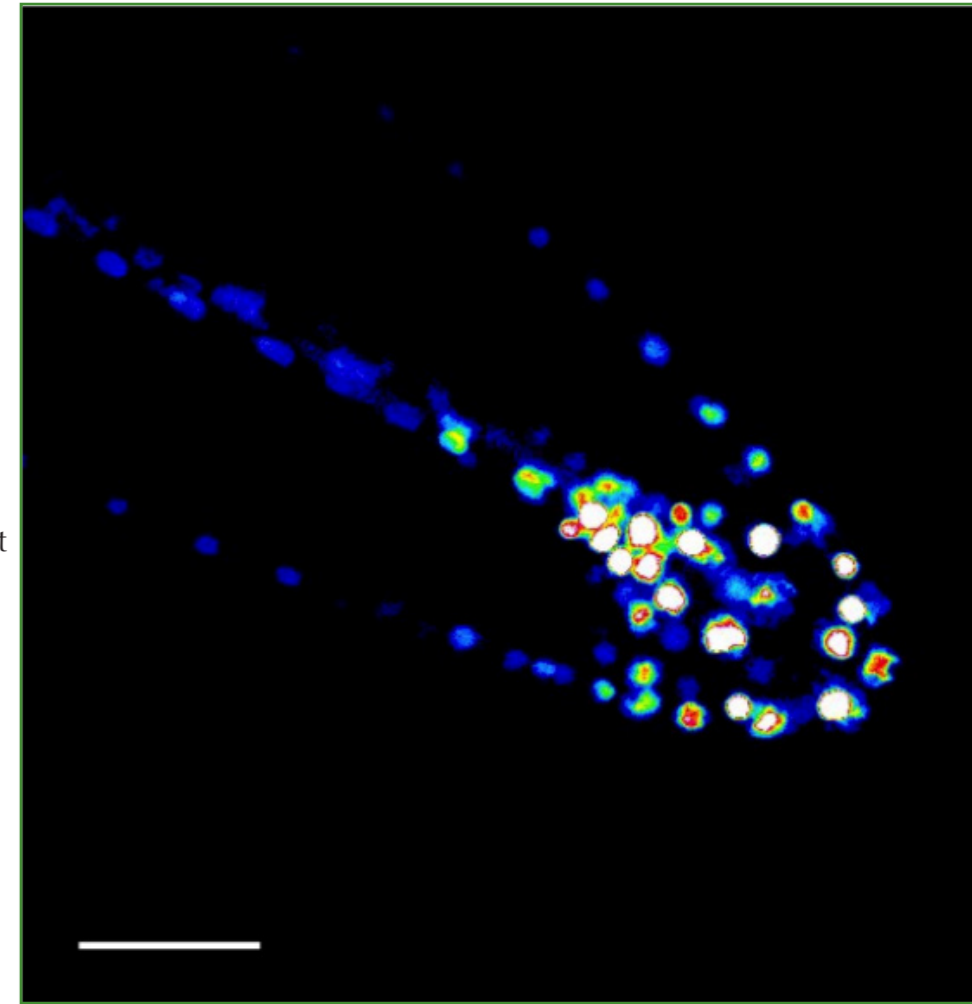
Our recent work has addressed the central question of how gravity-dependent non-vertical GSAs are set and maintained. We showed that non-vertical GSAs are sustained by means of an antigravitropic offset (AGO) mechanism that operates in tension with gravitropic response to allow stable angled growth. Further, we demonstrated that AGO activity requires auxin transport and also that auxin specifies the magnitude of the AGO in the gravity-sensing cells of lateral roots and shoots. The molecular mechanism underlying antigravitropic activity and how that activity is restricted to non-vertical branches is not known and is the subject of the study.

The project has two parts. In the first, we focus on the regulation of auxin transporters called PIN proteins that are responsible for moving auxin out of cells. In gravity-sensing cells, two particular PINs, PIN3 and PIN7, appear to mediate both gravitropic and antigravitropic auxin fluxes. The subcellular localisation and activity of PINs is regulated by their phosphorylation.

Our preliminary work has shown that manipulating the phosphorylation status of PIN3 and PIN7 is sufficient to alter the GSA of lateral roots, the simplest interpretation of these data being that antagonistic regulation at the level of PIN protein phosphorylation underpins or contributes to the maintenance of non-vertical GSAs.

This idea will be tested with a range of mutants and fluorescently labelled PIN proteins and PIN phosphoregulators in combination with advanced imaging techniques including vertical-stage super-resolution microscopy using instant structured illumination microscopy (iSIM) and Airyscan confocal imaging.

The second part of the project seeks to identify the molecular components that are required for the generation of non-vertical GSAs and, in particular, to understand how the gravity-sensing cells of non-vertical lateral organs differ from those in the main root–shoot axis. This will be achieved using a forward genetic screen for Arabidopsis mutants with altered GSA control in the root and/or shoot.



Kepinski: Arabidopsis lateral roots growing at their GSAs are characterised by balanced auxin response on the upper and lower sides of the organ, visualised here with the auxin response reporter DR5v2.

The ultimate aim of our work is to move beyond Arabidopsis to use what we have learnt during the project both to understand the wonderful variation in patterns of GSA control observed throughout nature and to develop new approaches to the improvement of water and nutrient uptake in crops via the manipulation of root growth angles.

 Celebrating Plant Development at the Inaugural Event of the Sainsbury Laboratory Symposium

Emily Marr,
Cambridge University



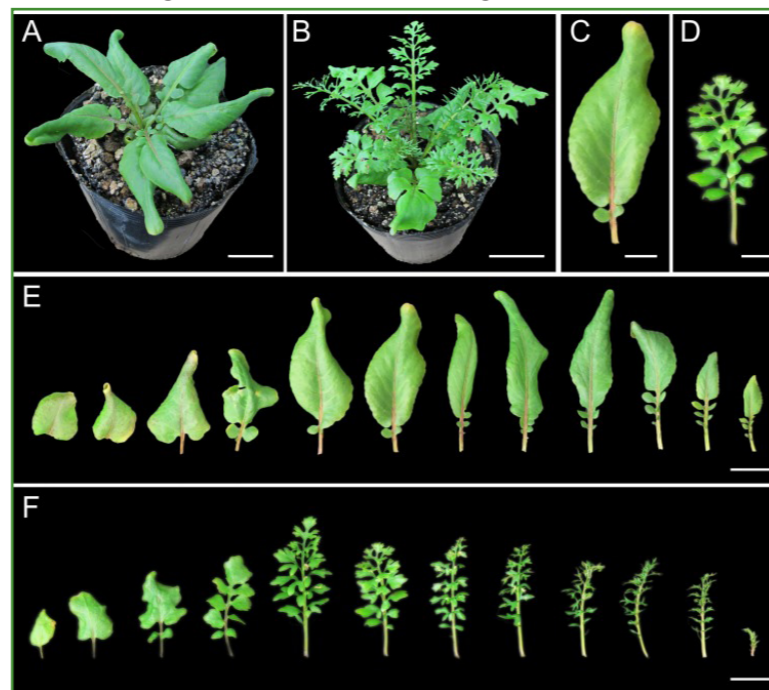
From the 4th-6th April 2016 the Sainsbury Laboratory Cambridge University (SLCU) held its inaugural symposium. In line with the research focus of the institute, scientists from around the world were invited to discuss their current work on the regulatory systems involved in plant growth and development. The event comprised of 18 talks and 36 posters for over 110 guests to enjoy.

As sessile organisms, plants need a highly developed signalling system that integrates developmental and environmental stimuli to ensure an appropriate response. This signalling system must be effective from the point of seed-set through to the maturity of the plant. It coordinates successful germination, subsequent growth and later development. Besides responding to abiotic factors such as light, temperature and nutrient availability, plants must detect biotic factors, whether these are beneficial symbionts or harmful pathogens. Furthermore, the conditions experienced by a plant during its lifetime can affect the fitness of its progeny thanks to epigenetic inheritance. Throughout the symposium each of these aspects of plant life were addressed.

A single signalling molecule can have various roles in a plant and the final effect is often determined by a combination of factors. For

example, the opening keynote address by Prof Dr Sofie Goomachtig (Ghent University) explored the role of the phytohormone strigolactone in root development, yet highlighted that strigolactone has many other effects such as the inhibition of shoot branching, induction of hyphal branching in arbuscular mycorrhizal fungi and germination of parasitic plants. The effect of strigolactone depends on specific combinations of protein expression and localisation. For example, Dr Thomas Greb (Heidelberg University) spoke of the eight identified *Arabidopsis* *SMXL* genes encoding repressors of strigolactone signalling and specified that the expression of *SMXL4* and *SMXL5* in phloem-related tissues reflects their role in carbohydrate metabolism.

The architectures of plants are diverse and are shaped by the environment. Leaf shape is one of many examples of this diversity, with shapes including oval, lanceolate or sagittate. It is clear



Temperature dependent development in *Rorippa aquatica*.
From PLoS ONE, DOI: 10.1371/journal.pone.0141247.

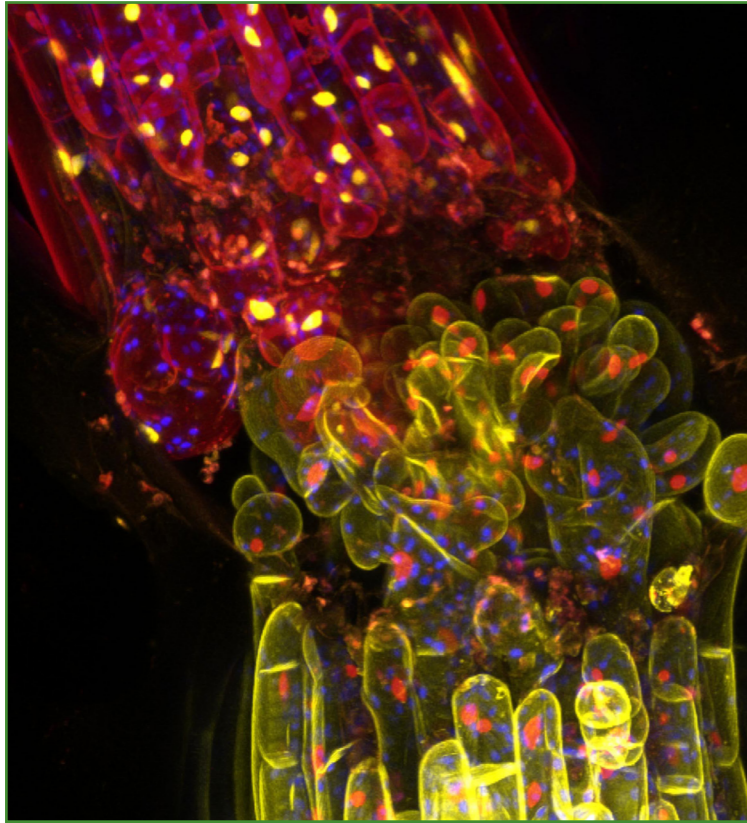
that different species of plant display different leaf forms. However, even the same plant can develop different forms in response to external stimuli. Prof Dr Seisuke Kimura (Kyoto Sangyo University) reported that in a terrestrial environment, *Rorippa aquatica* develops simple (undivided) leaves. However, new leaves that grow after transfer to an aquatic environment, are compound (divided into leaflets), a form that is better adapted for the absorption of dissolved CO₂. Temperature also affects leaf shape and actually overrides the other effects, such that *R. aquatica* grown in warm water develops simple leaves.

Plant architecture can be influenced by light. Surprisingly it is not only the above-ground parts of the plant that respond to light but also the roots below ground. The poster by Dr Erin Sparks (Duke University) revealed that light signalling represses development of underground roots in *Arabidopsis* but promotes the growth of stem-born roots that grow above ground in species like maize. In the final session of the symposium, Dr Kerry Franklin (University of Bristol) and Dr Christian Fankhauser (University of Lausanne) talked about the influence of UVB, red and far-red light on the morphogenesis of aerial organs. These light signalling pathways illustrate regulatory hubs where cross-talk occurs between light, temperature, the circadian clock, phytohormones and cellular factors. As shade avoidance can reduce a plant's stability and therefore limit crop density, an understanding of the molecular mechanism of this behaviour has direct implications for horticulture.

The plasticity and adaptation of plants within and between generations can be attributed in part to epigenetics. Epigenetics can be defined as changes in the expression of DNA without modification to the DNA sequence. While epigenetic inheritance is known to be rare in humans it is relatively common in plants. If a parent plant is exposed to particular environmental conditions, it is likely that the progeny will also be exposed during their lifetime. Thus, epigenetic inheritance primes the progeny for anticipated exposure.

An elegant example of the alternation of epigenetic inheritance across generations was provided by Dr José Gutierrez-Marcos (University of Warwick) in his description of salt tolerance. Plants which are initially intolerant to salinity and are grown in saline conditions give rise to progeny with acquired tolerance. If these progeny are grown in saline conditions, the subsequent generation will also be tolerant. If, however, the salt-tolerant progeny are grown in normal conditions, the subsequent generation will not inherit epigenetic salt tolerance.

Dr Gutierrez-Marcos also highlighted the significance of clonal propagation for pathogen resistance: depending on the original tissue from which a plant is propagated and the method of organogenesis induction – using hormones or zygotic factors – the plant will have a different epigenome, microbiome and resistance to pathogens. Equally, there are implications for seed germination in agriculture as the length of dormancy is negatively correlated with the temperature experienced by the parent plant.



Charles Melnyk: An Arabidopsis graft junction in the hypocotyl visualised using confocal microscopy of fluorescently labelled cell membranes and nuclei.
<http://www.slcu.cam.ac.uk/directory/melnyk-charles>

Dr Dana MacGregor (John Innes Centre) was the winner of the poster competition. She showed that the cooler the temperature experienced by the mother plant before seed set, the longer the dormancy period of the seed. Dormancy is affected by seed coat permeability and Dr MacGregor has been using genome-wide association studies to identify putative regulators of seed dormancy and permeability.

The runners-up of the poster award were Dr Charles Melnyk (Sainsbury Laboratory, University of Cambridge) and Jordan Brown (University of Sheffield). Dr Charles Melnyk presented the auxin-dependent developmental processes

involved in grafting, from the initial reconnection of the phloem, to the resumption of root growth and xylem reconnection (see figure). Jordan Brown presented her work on the influence of light and CO₂ on the stomatal development pathway and showed that the light receptor, phytochrome B, regulates plant water use efficiency in a CO₂-dependent manner.

This article has covered the broad themes of the symposium yet does not do justice to the vast number of posters and presentations, all of which were of high quality merit. Among other things, we learned that a single 1 mm-long Hessian fly can kill an entire wheat plant in four days (Dr Jeff Stuart, Purdue University), that powdery mildew induces polyploidy in host mesophyll cells via endoreduplication (Prof Mary Wildermuth, University of California) and that decoding the Ca²⁺ signal for the establishment of rhizobial symbiosis involves a combination of protein activity, resonance detection and counting of Ca²⁺ spikes (Dr Richard Morris, John Innes Centre). International gatherings such as this inaugural symposium held at the Sainsbury Laboratory are valuable for the sharing of ideas.

For me it was a successful and enjoyable event, which undoubtedly contributed to the majority of attendees improving their knowledge of current research into plant developmental signalling and hopefully generated a number of exciting collaborations.



Trait Introducing the Brassica Information Portal: a Web Repository for Brassica Data.

Annemarie Eckes and Wiktor Jurkowski,
The Genome Analysis Centre

As a community resource, the Brassica Information Portal (BIP) is a phenotype repository for Brassica trait scoring experiments. It is important to understand and create diversity in Brassica crops for further breeding of varieties. Therefore, the BIP will be a valuable resource for managing the data associated with these pre-breeding experiments that are focussed on discovery.

BIP is an open access and open source project, built on the schema of the former CropStoreDB (Love et al., 2012). New interface-based or programmatic submission/retrieval has been developed to be user-friendly for non-expert scientists and breeders. Importantly the BIP opens up opportunities to apply meta-analysis to data generated in the Brassica community.

Future aims for the development of this resource include the extension of previously built analytics tools to aid further integration by cross-linking with other sources such as COPO, CyVerse and

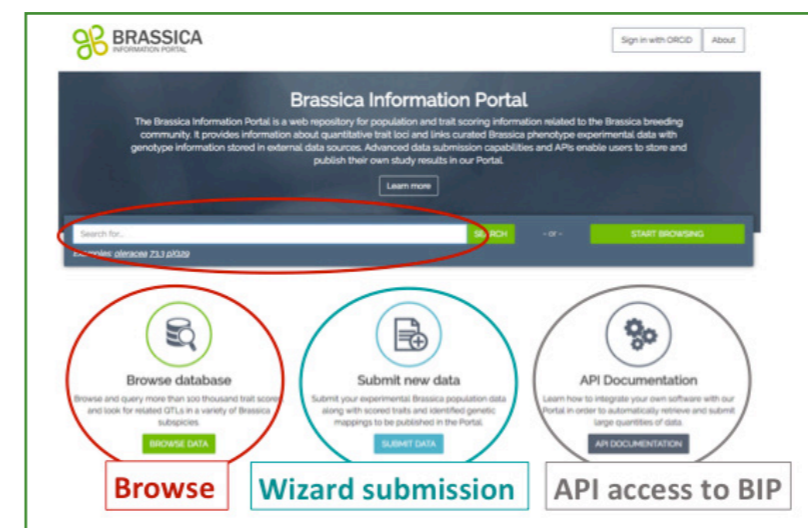


Figure 1: The BIP interface

EnsemblPlants. The Brassica Information Portal can be accessed at <https://bip.tgac.ac.uk/>. The source code is openly available on <https://github.com/eSpectrum-IT/brassica>.

The Brassica Information Portal (BIP) helps the Brassica Research Community to enhance their capabilities in data-sharing and integration with all its benefits. For example, readily available data from the database can serve 1) as evidence for new claims, 2) to identify knowledge gaps, 3) to recap existing knowledge, 4) to bridge between subfields, or 5) to help with selecting research directions.

The Brassica Information Portal offers a user-friendly interface for easily browsing the database. Its unique features are a login using ORCID, a DOI for each dataset, and easy wizard-based and programmatic submission and downloading of data.

Browsing the Database

In order to search the database, you can begin at the initial interface on bip.tgac.ac.uk (Figure 1), where a global search field lets you find any element in the database carrying your search query (e.g. query a trial name, a species or a trait descriptor). Alternatively, you can browse the database by clicking "browse database", and from

here you can navigate between the visible table interfaces: Populations, Traits, Trials, Linkage maps, QTL and Marker Assays. Inside these tables, you can further specify your queries by using the search fields on the top left corner of the displayed tables. This helps to select for specific elements of the table before downloading a .csv spreadsheet of the information displayed.

Submitting new Data

Browsing the database can be done without signing in but in order to submit to the BIP,

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BIP: The Brassica Information Portal

you need to sign up, facilitated by use of your ORCID account. Before submitting new trait scores (which are the values you obtain by trait measurement), you need to make sure that all your lines and varieties used in the experiment are registered in the database. For that, you can submit an experimental population by following the “Populations Submission” button. It is a straightforward wizard-based, four-step submission process concluded by entering metadata about the ownership and origination of the population (see submission steps, Figure 2A).

Once this is done, select “Trait Scoring Submission”. There, the wizard guides you through the four submission steps to submit your trait scoring data. You will be asked to select traits from an existing list. Should your traits not be currently defined in the database, you can create these traits manually. Once this is done, a .csv submission template can be downloaded, in which you can copy and paste your measured values and corresponding metadata on your experimental setup before re-uploading the file (see submission steps Figure 2B).

API Access to the Database

The BIP is one of the first phenotypic databases that can be accessed via an API. This means that data can be both uploaded and queried programmatically instead of using the wizard. This has the advantage that, when uploading data, standard metadata information can already be programmed in by default, which reduces submission time. Another advantage is the download process using the API. In this case GET

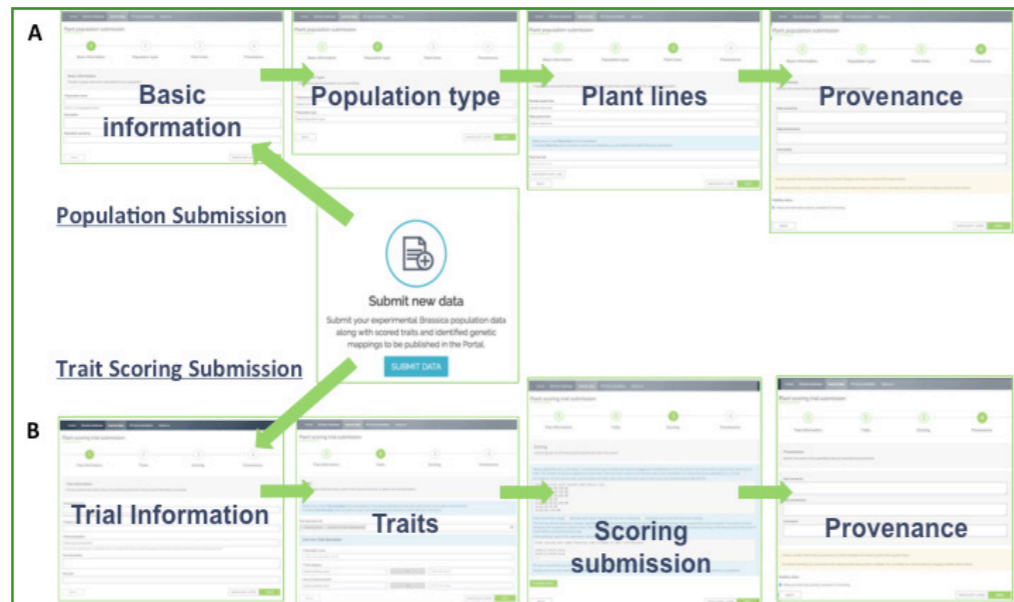


Figure 2: Submission steps.

comprehensive or specific depending on user needs. These queries can then be outputted in .json format, or automatically fed into analytic tools or workflows that run in, for example, Python. Detailed API documentation describing each field in the database can be accessed from the initial interface.

Contact

We would be very happy if you could try out the portal and encourage you to give us feedback on your experience. You can reach us on bip@tgac.ac.uk. Please, also follow us on Twitter [@BrassicaP](https://twitter.com/BrassicaP). The Portal is accessible from <https://bip.tgac.ac.uk/>. We thank the RIPR Project, funded by the BBSRC for financial support.

Finally if you need convincing as to all Brassicas are brilliant, not just Arabidopsis, have a look at our recent blog post: <http://blog.tgac.ac.uk/brilliant-brassica/>.

Sources

Love et al (2012), InterStoreDB: A Generic Integration Resource for Genetic and Genomic Data. *Journal of Integrative Plant Biology*, 54: 345–355. doi: 10.1111/j.1744-7909.2012.01120.x.

GARNish

UKPSF Annual Meeting

UKPSF Annual Meeting
Report: April 2016

Geraint Parry,
GARNet Coordinator



The UK Plant Science Federation (<http://www.plantsci.org.uk/>), as I hope most readers will know, is a grouping of organisations who have an interest in promoting plant science in industry, research and education through the UK and beyond. It was established in 2011 and currently has over 30 member groups, ranging from The Biochemical Society through to the British Ecological Society. The inaugural annual meeting took place in 2011 at the John Innes Centre and so in the fifth year the PlantSci2016 meeting (<https://plantsci2016.org.uk/>) returned to this worldwide centre for plant biology.

A glance through the schedule (<https://plantsci2016.org.uk/home/programme/>) for the two-day meeting highlights a fundamental challenge that the UKPSF faces, namely catering for the enormous breadth of interests found within its membership. The topic of the meeting

was ‘Plants in a Changing World: Molecule to Ecosystem’ and it truly covered all bases with talks on topics as diverse as ‘The wheat epigenome’ through to ecological studies of chalk grasslands. Each session was supported by different member organisation, which surely aided with the planning of such a broad set of sessions. Overall attendance was approximately 80 delegates but there was certainly some talk selection-bias, with people seemingly avoided sessions that weren’t directly relevant to their research.

Regardless of topic, each of the talks were of very high quality with the initial keynote session provided by Professor Giles Oldroyd from the host institution. He described his lab's involvement with Engineering Nitrogen Symbiosis for Africa (ENSA) (<https://www.ensa.ac.uk/home/>), an incredibly ambitious project that aims, ultimately, to move the components of the nitrogen fixation pathway into cereals. As Professor Oldroyd stated, even a moderate amount of N fixation could be life-changing technology for subsistence farmers in sub-Saharan Africa. Personally, having seen a number of talks about ENSA over the past few years, it was notable that the tone of the talk had



The conference was hosted at the John Innes Centre. Photo: <http://blog.plantsci.org.uk/>

subtly changed. It is no longer purely a description of the grand goals of the research but now documents the day-to-day achievements that the project is making.

In recent times the project has linked with another Gates Foundation-funded project, C4R (<http://c4rice.irri.org/>) and RIPE (<http://ripe.illinois.edu/>) to use the Golden Gate cloning method to modularise construct building. This allows the creation of an enormous number of constructs for the analysis of gene expression and function. Currently, the bottleneck in this system involved effective plant transformation so, in collaboration with the BRAC project (<http://www.bract.org/>), they are starting to use a system of destructive screening in barley for rapid assessment of gene expression. Professor Oldroyd believes that future work will involve this industrialised molecular construction that is based around the principles of synthetic biology. There is no doubt that this keynote was an outstanding prelude to such a broad-based meeting as it spanned many scales, from large-scale industrial molecular construction through to real world solutions surrounding food insecurity.

The first session was organised by Richard Flavell and sponsored by The Genetics Society (www.genetics.org.uk), providing an excellent range of talks about the 'hidden' regulatory world that surrounds epigenetic genome regulation, the mechanisms that drive heterosis, and finally, an exciting talk from Sir Professor David Baulcombe. Sir Baulcombe presented his lab's recent findings on the biology of small RNA regulation. In his summing up, Professor Flavell reminded us that control of phenotype is far more complex than the linear information held within a genome sequence!

The second session was organised by Dr Daniel Gibbs, sponsored by the Biochemical Society (www.biochemistry.org), and was entitled 'The

molecular basis of signal transduction in plants'. This included descriptions of different aspects of cell signalling from younger faculty members including Eirini Kaiserli and GARNet committee member Steven Spoel.

Arguably the most exciting hour was provided by the five excellent talks from the 'Future Generations' session, delivered on subjects including control of arsenic uptake (Emma Lindsay), the synthetic production of medicinal curry (Keir Bailey), exploring floral heteromorphy (Jonathan Cocker) and bioinformatic approaches to find resistant genes (Jan Bettgenhaeuser). However, in this case there had to be a winner, so the secret audience ballot decided that Emily Hawkes from the JIC was the prize-winner for her talk describing non-coding RNAs in the control of flowering time. A well deserved victor!



Emily Hawkes presented her prize by Dr Celia Knight
 Photo: <http://blog.plantsci.org.uk/>

After a pleasant evening of food, wine and chat the second day kicked off with a keynote provided by Professor Phillip Poole (University of Oxford), who has developed *in vivo* luminescent imaging of the interaction between roots and soil microorganisms. Subsequently, the first main session of the day focused on the plant microbiome with Alison Bennett (James Hutton Institute) and Gabriele Berg (Graz University) explaining how particular types of microbe

interact with plants. The session was ended by Nik Cunniffe (University of Cambridge) who documented his work modelling the transmission of Sudden Oak Death disease across California. Sadly his message wasn't so positive: even by taking precautions now, the spread of the disease is inevitable. However, lessons learnt from this outbreak will help plan future avoidance strategies.

The British Ecological Society (www.britishecologicalsociety.org) sponsored a session on 'Ecological Resilience' chaired by Dr Alan Jones and featuring a couple of talks from Bangor Environment Centre looking at the impact of flooding on either the whole environment scale or in a lab context. Antonio Sanchez-Rodriguez described the effect of extreme flooding under different light conditions on plant-microbe interactions, showing that flooding in the dark favours the interaction with anaerobic bacteria. Sadly, he also showed that earthworms don't do so well in flood conditions, whether it is light or dark.

The final session focused on abiotic stress and was sponsored by SCI Agrisciences Group (<http://www.soci.org/membership-and-networks/technical-groups/agrisciences-group>). The session greatly benefitted from the speakers giving a wider perspective on the translational aspects of plant science research and what is possible when academics interact with industry.

Mafalda Nina from Syngenta described how they have collaborated with Sean Cutler (UC Riverside) to undertake chemical genetic screens to discover agonists of the ABA receptor. This has led to the development of new seed treatments aimed at regulating germination rates.

The topic of seed treatments was also discussed by Steve Adams from Plant Impact Inc. (<http://www.plantimpact.com/>), who has worked with

researchers at Lancaster University to develop stress resistance in plants following seed hormone treatments. During the Q&A Dr Adams explained that this type of translational interaction can have its genesis from either side; sometimes academics will approach companies but also he explained that companies do spend time searching the literature for new discoveries that may have translational applications.

Richard Haslam from Rothamsted Research described work to identify novel stress loci by genome comparison between Arabidopsis and Eutrema. In the Q&A Professor Haslam mentioned that over the past few years Rothamsted has made a concerted effort to increase their levels of public engagement to, in particular, ensure that GM trials are viewed more favourably.

Improving communication was a theme that ran through the meeting and included a lunchtime session led by Harriet Truscott from the Gatsby Plant Science Education team. Here, participants were encouraged to discuss the strategies that they have used for public outreach, highlighting both what does and does not work! In that session Dawn Arnold (UWE) gave an interesting insight on the benefits of working with a specialist science communicator, as those types of skills may not be as intuitive as many scientists feel they are! On that related topic, there was plenty of social media activity at the meeting so if you want to learn about what was 'said' in real time, please check out the Twitter hashtag [#PlantSci2016](https://twitter.com/PlantSci2016).

Overall the conference was well put together. It remains to be seen whether future meetings will be as broad in scope as this version or whether a more focused topic will be chosen. Clearly, this might be determined by whoever is willing to organise the meeting and whether there is funding available for a particular focus area. Hopefully the results of that will appear in April 2017!

AtRTD2: a new transcriptome for Arabidopsis

John Brown, Runxuan Zhang, Cristiane Calixto and Hugh Nimmo,
University of Dundee, James Hutton
Institute and University of Glasgow

Groups at the University of Dundee/James Hutton Institute, the Universities of Glasgow and Vienna and other colleagues have released AtRTD2, a new transcriptome reference for Arabidopsis (Zhang et al., 2016). The objective of making the Reference Transcript Dataset (AtRTD2) was to exploit the accuracy of transcript quantification of programmes such as Salmon and Kallisto for the analysis of Arabidopsis RNA-seq data for isoform/gene expression and, in particular, alternative splicing (AS).

Analysing our RNA-seq data using an earlier version of the RTD already demonstrated the value and potential of this approach and the need to have the best possible transcriptome to capture the diversity of transcript isoforms. This approach contrasts the analysis of single RNA-seq datasets using the current standard transcript assembly pipelines, which fail to deliver such diversity and accuracy. We therefore constructed AtRTD2 (Figure 1), which assembled 8.5 billion pairs of reads from 129 RNA-seq libraries of wild-type plants, mutants and over-expression lines with different treatments.

The transcriptomes were merged with our initial RTD and Araport11 (described in GARNish24, December 2015 and by Agnes Chan and Chris Town on page 32 of the present issue). Quality control criteria were applied to overcome the

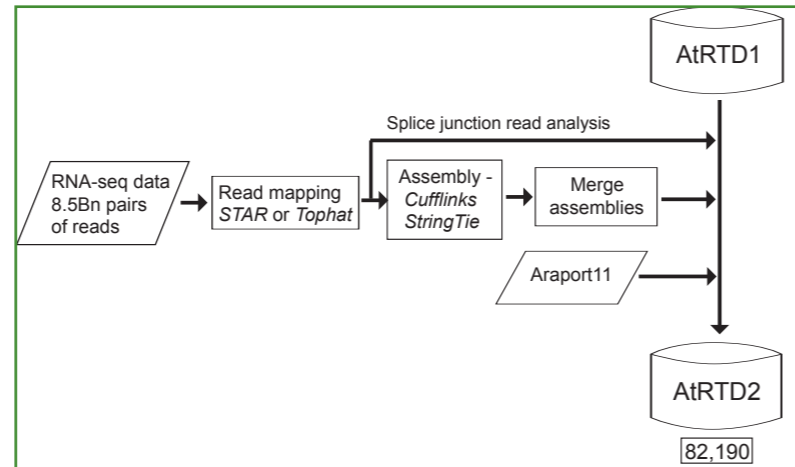


Figure 1. Assembly of AtRTD2. New RNA-seq data was assembled using different programmes and splice junction reads used to remove unsupported transcripts from AtRTD1 before merging with new data assemblies and Araport11. Quality control filters were applied at all stages (see Zhang et al. (2016).

many errors of assembly programmes and to remove, for example, redundancy and transcript fragments which affect accuracy of quantification. AtRTD2 contains over 82,000 unique transcript isoforms. It is unlikely as yet to be complete or 100% accurate, but as the most diverse, supported set of transcripts available, it will be a valuable addition to Araport11.

Extensive validation to test AtRTD2 by analysing our RNA-seq datasets with Salmon and comparing splicing ratios derived from RNA-seq and high resolution RT-PCR showed good correlation, except for genes with >1 transcript with considerable variations in the 5' or 3' UTR regions where quantification could be poor. We overcame this problem by modifying the RTD to produce AtRTD2-QUASI for Quantification of Alternately Spliced Isoforms. AtRTD2-QUASI significantly improved transcript quantification compared to other Arabidopsis transcriptomes with fewer (and therefore missing) transcripts and which do not correct for UTR variation.

Using Salmon/AtRTD2-QUASI to analyse a time-course of plants transferred from 20°C to 4°C, we can dissect individual transcript expression and the effects of low temperature on AS (Figure 2). From initial analysis, several thousand genes have significant AS including over 1,000 that are regulated only at the AS level (no change in overall gene expression levels) and represent novel genes not previously associated with the cold response. Therefore, re-programming of the transcriptome in response to lowering temperature occurs at the level of both transcription and alternative splicing.

So why use AtRTD2 and AtRTD2-QUASI?

Major changes in transcription and AS will occur during development and differentiation, and in response to abiotic and biotic stresses. RNA-seq analysis using rapid and accurate tools such as Salmon and Kallisto, along with the most comprehensive RTD, will capture

transcript-specific information to provide more complete pictures of the dynamic transcriptome and underlying regulatory mechanisms in high resolution. The transcript abundance output from these programmes can feed into differential expression programmes (e.g. Sleuth) and alternative splicing analysis programmes (e.g. SUPPA, which we are currently improving for plants) and provide accurate information of transcriptional and AS changes. The same approaches can be applied to new model plants and, more importantly, to crop species – all you need is a good RTD!

AtRTD2 and AtRTD2-QUASI are available at: <http://ics.hutton.ac.uk/atRTD/> and will be incorporated into Araport11.

Zhang et al. (2016) bioRxiv doi: <http://dx.doi.org/10.1101/051938> May. 6, 2016.

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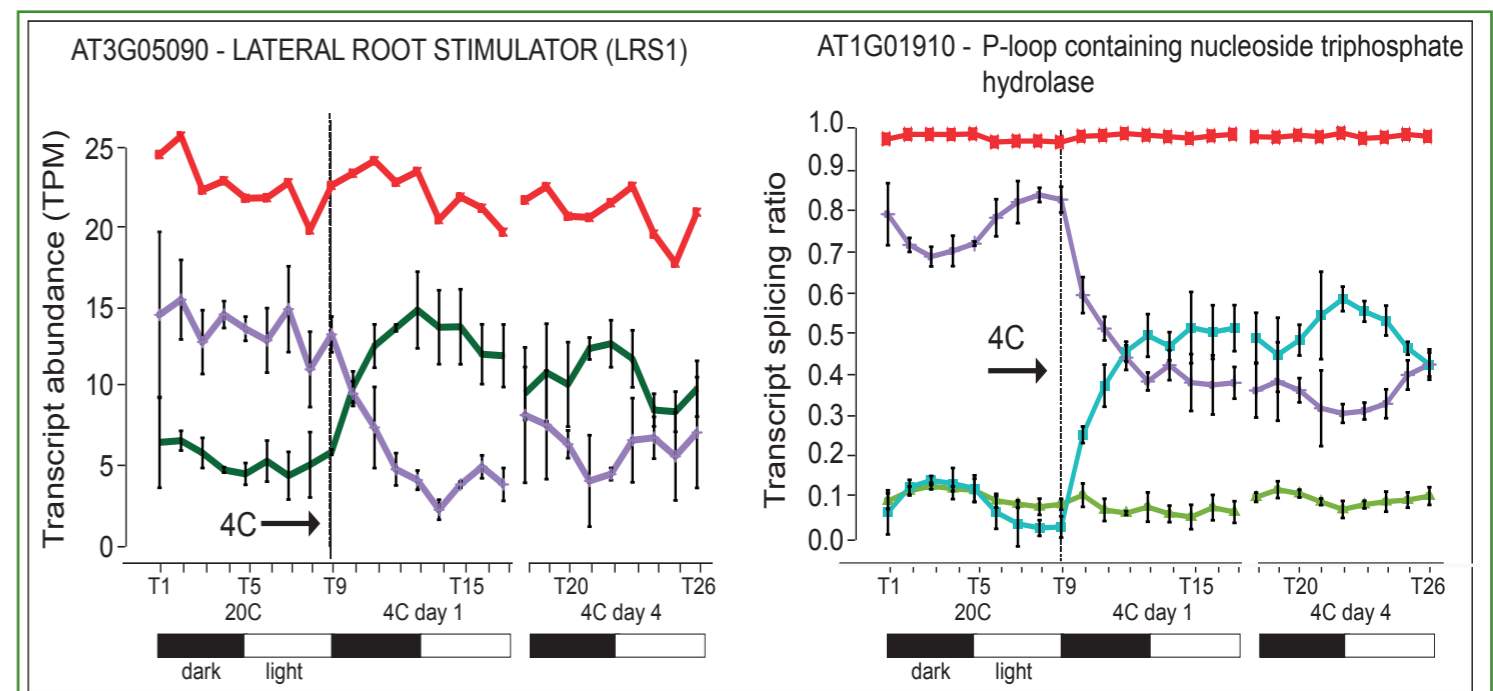


Figure 2: Low temperature-induced AS and isoform switches. Transcript-specific profiles across 26 time-points of plants transferred from 20°C to 4°C. Left – transcript abundance plot in transcripts per million (TPM); Right – transcript splicing ratio (=Transcript TPM/ \sum TPMs for gene). Coloured lines represent individual transcript isoforms except red (total expression).



Spotlight on: The Sainsbury Lab, Cambridge University

Kindly compiled by Siobhan Braybrook
and Phil Wigge

The Sainsbury Laboratory, University of Cambridge, is a Research Institute whose aim is to obtain an integrated understanding of plant development. With questions ranging from how pattern is generated by meristems, how environmental factors modulate growth and development, how hormones move and are sensed within tissues and cells, how regulatory circuits topology governs signalling dynamics to how growth causes movement in plants – we aim to understand the complex and dynamic processes that shape a plant during its lifetime. We are a collection of research groups from diverse scientific backgrounds who work together in a collaborative environment to further our understanding of plant development by using multi-disciplinary and multi-scale approaches.

The study of plant development is being transformed by the new scientific and technical resources becoming available to biologists, including high-throughput DNA sequencing, new imaging methods, increasingly sophisticated genetic tools, and refined chemical interventions. The data derived from these approaches have opened the way for predictive computational models, which are essential for understanding the dynamic, self-organising properties of plants. We now have an unprecedented opportunity to obtain an integrated understanding of plant development, setting the stage for a new synthesis that will draw on molecular, cellular, whole plant, and population biology to elucidate how plants are constructed.

Professor Ottoline Leyser, Director and Group Leader

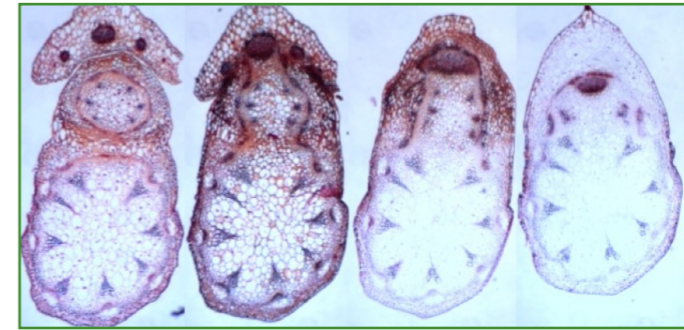
Our research is aimed at understanding the role of plant hormones in plant developmental plasticity, using the regulation of shoot branching as a model. Axillary meristems, which are established in each leaf axil formed from the primary shoot apical meristem, can remain dormant or activate to produce a branch.



Ottoline Leyser

The decision to activate or not involves integration of diverse environmental, physiological and developmental inputs, and is mediated by a network of interacting hormonal signals that generate a rich source of systemically transmitted information, which is locally interpreted to regulate branching. At its hub is the polar auxin transport system, which extends throughout the plant, transporting auxin basipetally from shoot apices to the roots. The system is dynamically modelled and remodelled by auxin itself.

Our current data suggest that shoot apical meristems compete for common auxin transport paths to the root. Other hormonal signals can influence branching by modulating the auxin transport network and/or the ability of buds to compete for access to it. For example, strigolactones can reduce the accumulation of auxin transporters at the plasma membrane thereby increasing competition between branches. We are working to understand the dynamic properties of this system and their implications for adaptive developmental plasticity.



Leyser: Serial sections through an *Arabidopsis* inflorescence across a cauline node bearing an active branch

Professor Henrik Jönsson, Associate Director and Group Leader

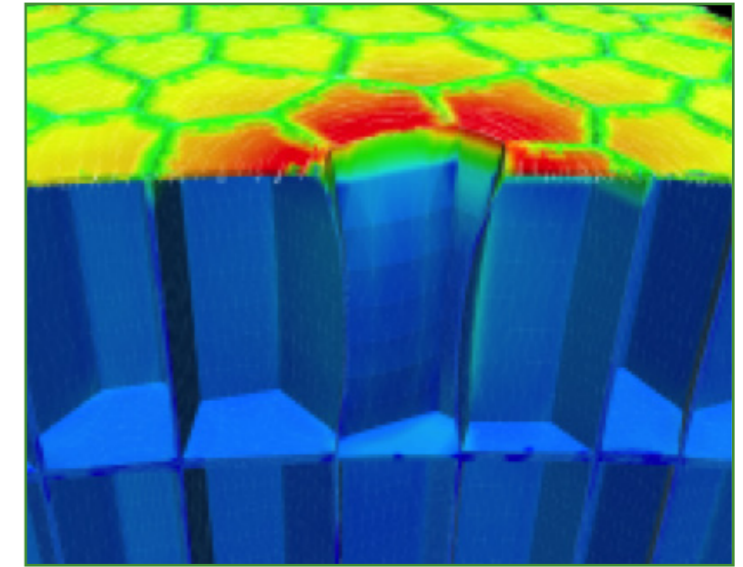
Our research group is focused on understanding differentiation via gene regulatory interactions together with hormone signalling and the development of cellular and organ shapes via mechanical interactions. We use a Computational Morphodynamics approach, where mathematical models are integrated with live imaging at single cell resolution. Our main interests surround the developmental biology of plants. We try to understand how the stem cell niche, residing in the shoot apical meristem, can be maintained, both in terms of stem cell expressing genes and size and shape of the tissue.



Henrik Jönsson

The spectacularly symmetric patterns of organ formation at the meristem, phyllotaxis, is another problem we study by combining molecular and mechanical interactions. We also study similar processes in leaf, flower, and root development.

Another avenue of our research is the study of single cell behaviour within plant tissues and in protoplasts. The overarching objective is to develop an integrated understanding beyond the



Jönsson: A computational simulation of a 3D plant tissue showing stress distributions after ablation of one cell.

single-mechanism description currently dominating the research field.

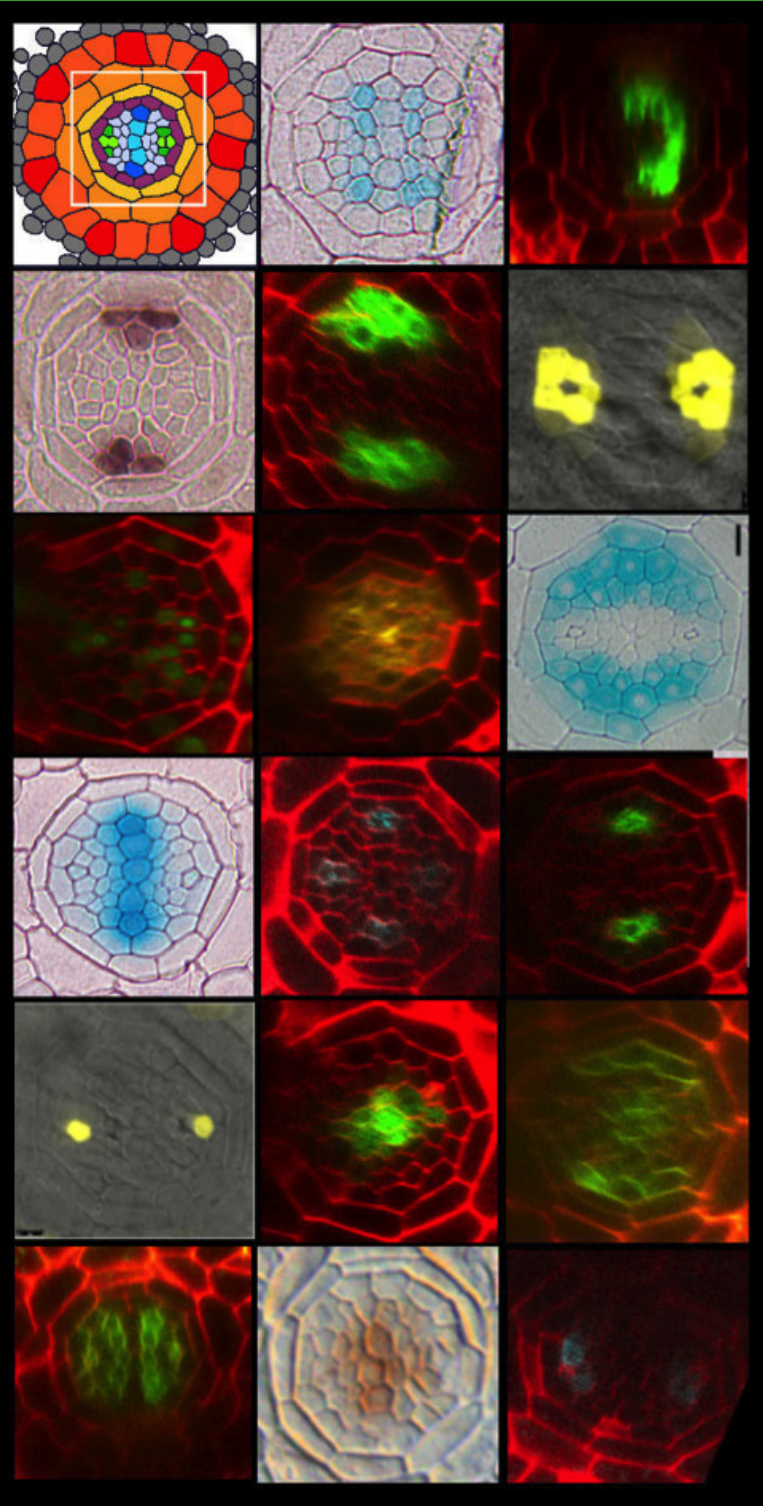
Professor Yrjö Helariutta, Group Leader

Our research is focused on understanding how a plant stem cell population, the vascular cambium, is genetically controlled to pattern and promote the formation of the vascular system. We are investigating this process during procambium/phloem development in the *Arabidopsis thaliana* root.



Yrjö Helariutta

During the early events of procambial patterning adjacent fields for auxin and cytokinin signalling are established for subsequent xylem and phloem development, respectively. We have recently identified a few novel regulatory factors that mediate the interaction between the two hormones. We have also identified a family of transcription factors that work downstream of the hormonal control.



Helariutta: The figure presents some of the various markers used in our research. The first panel is a graphical illustration of the various cell types within the *Arabidopsis thaliana* primary root. The following images are optical, plastic and paraffin cross sections at the meristematic region of primary roots analysed 5 days post-germination, cropped to show the vascular cylinder and endodermis. The majority of these markers are in wild type and some are in a mutant background.

Cell-to-cell communication events involving transport of different factors through plasmodesmata orchestrate the phloem formation and are important in understanding vascular development. Using a genetic screen, we discovered a mutation in a callose synthase gene which led to excess callose deposition at plasmodesmata. By expressing the mutated gene under various inducible promoters, we are currently analysing how accumulation of the callose polymer at the neck regions of the plasmodesmata blocks symplastic communication.

Dr. Phil Wigge,
Group Leader



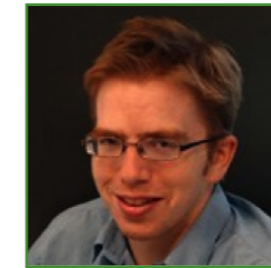
Phil Wigge

We are interested in how temperature is perceived by plants, and how these signals are integrated into development. Key questions being addressed by the lab include: 1) How is temperature perceived? To address this question we have carried out a genetic screen for components of the temperature perception pathway in *Arabidopsis*. This screen revealed the importance of H2A.Z-nucleosomes in mediating temperature responses on transcription.

2) What is the regulatory logic underpinning the floral transition? The floral transition is an important developmental decision, and temperature information is key for the correct timing of flowering. We are now analysing how warm temperature promotes flowering.

3) What is the temperature epigenome? The temperature transcriptome is tightly regulated by alternative nucleosomes containing H2A.Z, and we are analysing how these marks change in response to temperature.

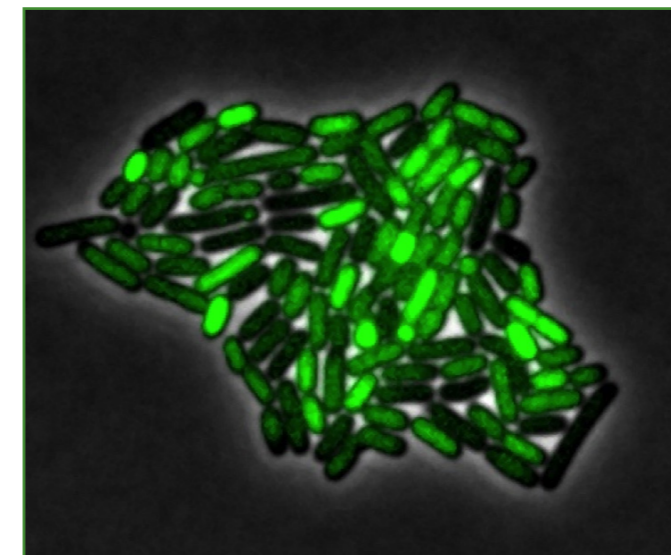
Dr. James Locke,
Group Leader



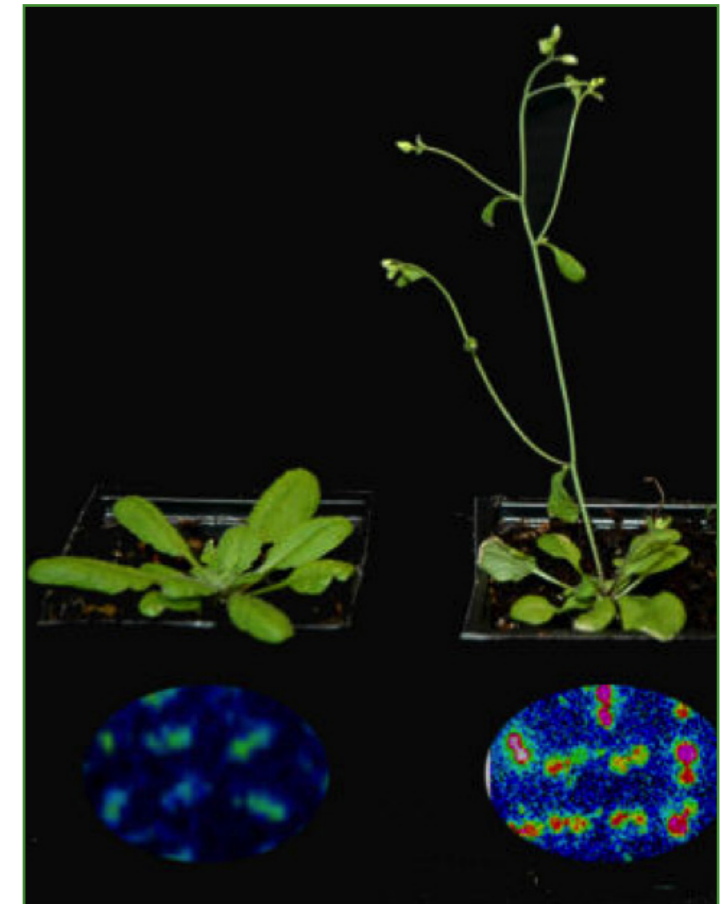
James Locke

Our main research interest is to gain a quantitative understanding of how cells respond to environmental signals. To do this, we observe cellular behaviour at the single cell level as traditional approaches that take an average from a population can obscure heterogeneous responses and novel dynamics. For example, by examining *Bacillus subtilis* gene expression at the single cell level, we have discovered that the alternative sigma factor sigB is activated in discrete stochastic pulses, a behaviour missed by previous bulk studies (see figure).

In addition, we focus on developing a quantitative understanding of signal integration and gene circuit dynamics at the single cell level in Cyanobacteria. Cyanobacteria, which rely on photosynthesis for metabolism, must anticipate day/night cycles, as well as respond to much faster environmental changes. By analyzing gene circuit dynamics at the single cell level in Cyanobacteria,



Locke: Snapshot of *B. subtilis* colony containing a fluorescent reporter for sigB (green) growing under energy stress (60 µg/ml mycophenolic acid, MPA). Note pulsing (bright) versus non-pulsing (dim).



Wigge: The *entr1* mutant (left) flowers early and has increased expression of HSP70:LUC

we are developing fundamental insights into how cells respond to diverse, dynamically varying inputs over multiple timescales.

Dr. Sebastian Schornack,
Group Leader

Our research aim is to understand the plant processes supporting microbial colonisation. Plants are host to numerous microbes. The symbiosis of legume roots with nitrogen-fixing bacteria and the widespread association of plants roots with phosphate-offering mycorrhizal fungi are well known examples of how plants take nutritional benefit from microbes. However, plants are also target of numerous disease-causing pathogens, often resembling in shape and lifestyle their beneficial cousins.

Working with the filamentous pathogen *Phytophthora palmivora* and arbuscular mycorrhiza fungi we study plant processes that enable general colonisation by microbes, and those which allow plants to discriminate between beneficial and detrimental microbes. We recently identified genes which are required for both types of vinteraction and genes which impaired symbiosis but actually promoted diseases. By knowing these genes and understanding the underlying processes opens up the possibility to modify them to support beneficial interactions, or to control a wide range of pathogens with similar lifestyles. An important factor is the degree of overlap with plant development. A good example is plant cell walls, which are a major barrier for pathogens but need to let symbiotic fungi into the plant cells to deliver phosphate. If we try and modify the plant cell wall then this is likely to impact shape and size of plant organs. Therefore, it is important to characterise a diverse range of colonisation processes to maximise our chances finding one that can be tweaked.



Sebastian Schornack

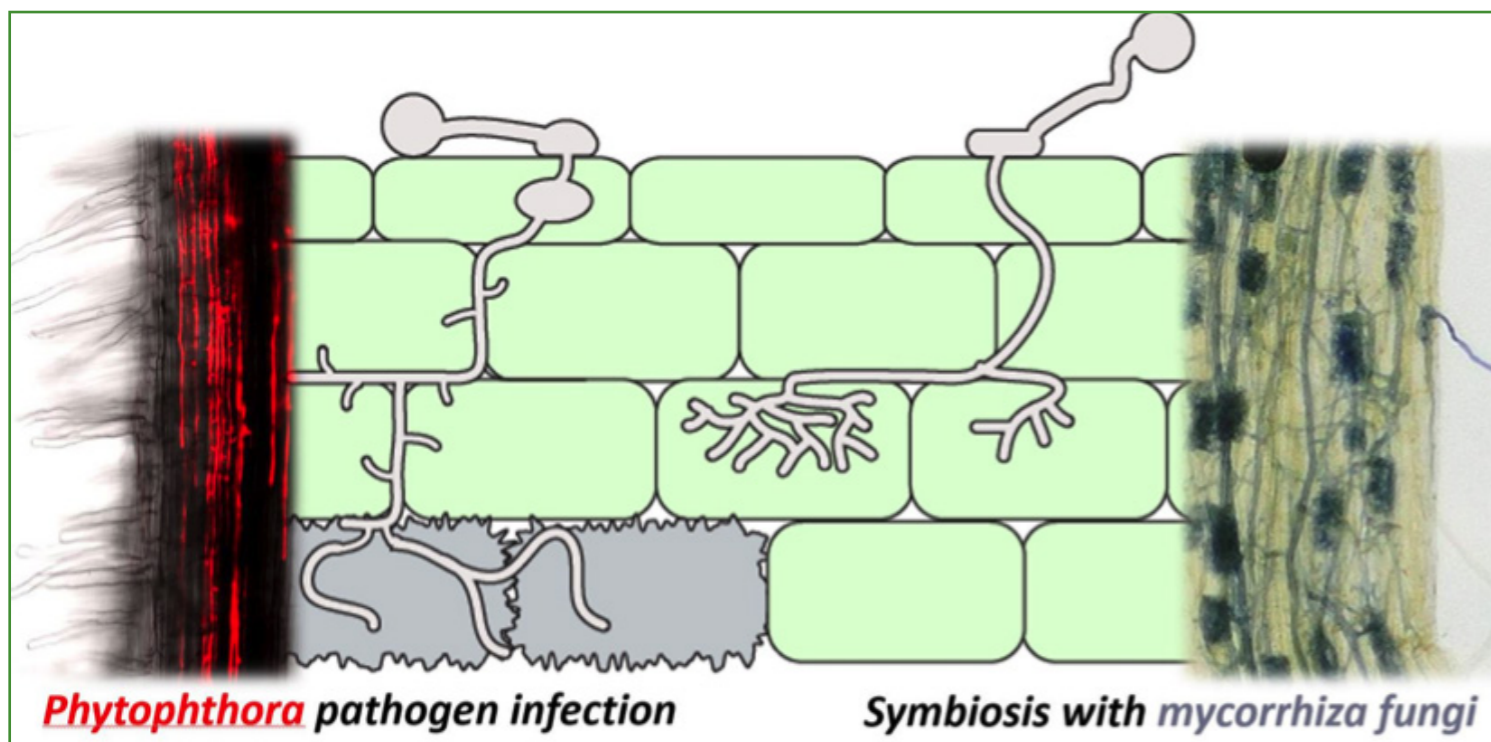
Dr. Jerzy Paszkowski,
Group Leader

Our current research focuses on the epigenetic regulation of transcription and on molecular mechanisms contributing to formation of transgenerationally stable epigenetic states (epialleles). This includes environmental and developmental inputs that have direct consequences for the heritable alteration of transcriptional states and contribution of retrotransposons to the genome-wide landscape of epigenetic regulation.

Recently, we revealed surprisingly selective epigenetic, environmental and developmental mechanisms controlling retrotransposition in *Arabidopsis*. We will now build on this knowledge and plan to establish a well-controlled retrotransposition system in a crop plant and liberate the innate genetic diversity buried in silenced TEs.



Jerzy Paszkowski



Phytophthora pathogen infection

Symbiosis with mycorrhiza fungi



Paszkowski: Nutty nut? Variation in leaf colour in a hazelnut tree possibly due to a transposon

Dr. Alexander Jones,
Group Leader

My research focuses on patterns and dynamics of plant hormones at high spatial and temporal resolution using recently engineered fluorescent biosensors expressed in living tissues.

Because each plant hormone is a potent regulator of a variety of developmental and physiological programs, their accumulations and depletions are tightly regulated in space and time. We are interested in how and why various signals integrate to modulate hormone biosynthesis, catabolism, modification and transport in order to achieve the hormone patterns and dynamics we observe with fluorescent biosensors.

The building of a systems level understanding of the where, when and how much of hormone levels and the discovery of novel regulators of hormone metabolism will augment crop improvement strategies targeted at traits that are regulated by hormones such as growth rates and stress tolerance.

We focus on the following questions: how much hormone is present in a given cell compared to its

neighbours? How about over developmental time or after a stress?

Professor Elliot Meyerowitz, Distinguished Associate

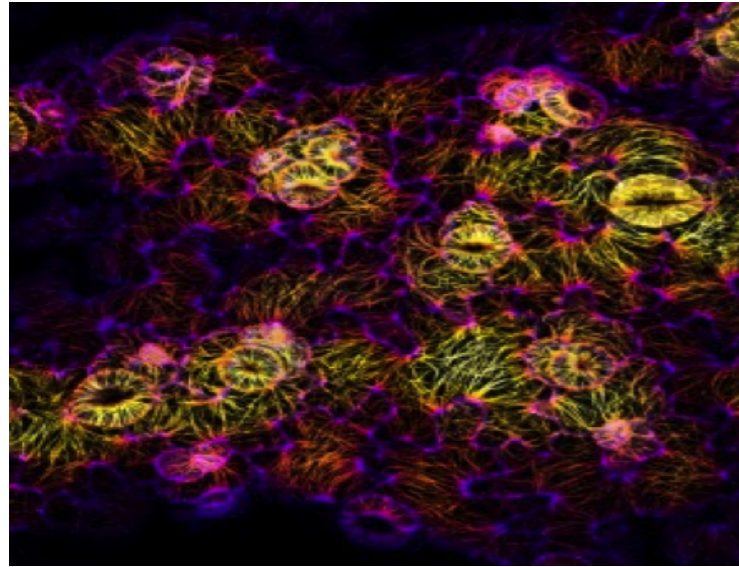
My research aims to understand the mechanisms of plant development, using both experimental and computational methods to test hypotheses. We concentrate on the shoot apical meristem and its derivative structures (primarily flowers), because this meristem is responsible for the development of the entire above-ground part of the mature plant, and utilises a number of different pattern-forming processes that are as yet poorly understood.

Our experimental organism is predominantly *Arabidopsis thaliana*, because of the ease with which genetic and molecular biological studies can be done using that model system. We also use other plant species in the laboratory when they offer an experimental advantage.

Our method of study is what we have termed Computational Morphodynamics – the combination of real-time live imaging to capture the dynamics of plant development, couching our hypotheses as mathematical models (so as to be absolutely explicit about assumptions and parameters), and then testing them computationally and experimentally. We concentrate on three processes in plant development - the development of flowers, the growth of shoots, and the *de novo* generation of shoot meristems during regeneration.



Elliot Meyerowitz



Meyerowitz: Microtubules in an Arabidopsis cotyledon epidermis.



Siobhan Braybrook

Dr. Siobhan Braybrook,
Career Development
Fellow

We are interested in understanding how shapes are grown in biological systems, specifically when a cell wall is involved, as

in plants and algae. In all organisms, the growing of a shape is a complex process requiring specific gene products, signalling, mechanical alterations, and coordination of cell growth.

Our group addresses this fundamental process in biology using a multidisciplinary approach including plant physiology, biochemistry, genetics,

molecular biology, differential geometry, materials science, and physics.

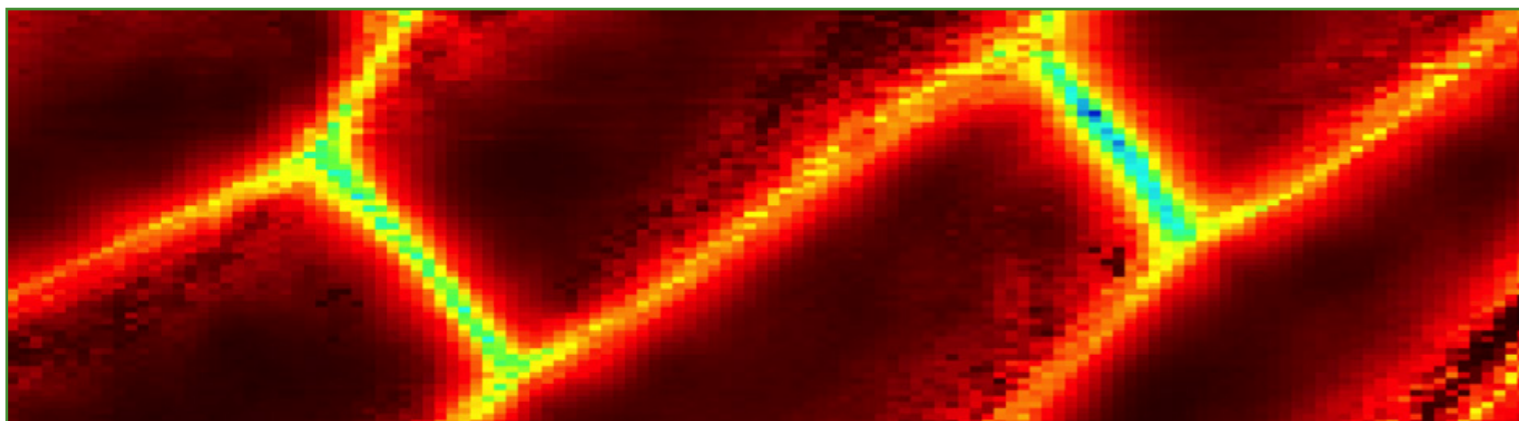
For a plant cell, the cell wall is the main structural element, controlling shape and growth of the cell and therefore tissues as a whole. Our group has three main goals: 1) to quantitatively describe shapes and growth, 2) to understand the mechanics of shape growth in plants, and 3) to understand the cell wall as a dynamic composite material.

In addition we investigate the mechanics of shape growth in plants. We utilise many different plant species and growth systems to understand how plants grow shapes. From mersitemes to hypocotyls, roots, and leaves in species such as tobacco, maize, Marchantia, sunflower, Arabidopsis, and the brown algae Fucus and Sargassum.

Dr. Katja Jaeger,
Career Development Fellow

I am interested in how plants control their development in response to a changing environment.

Sensing, integrating and remembering environmental information allows plants to make key life-cycle decisions at the right time to optimise their fitness. These questions are of interest from both a fundamental scientific perspective as well as having potential application



Braybrook: A map of cell wall elasticity in the elongating hypocotyl epidermis in Arabidopsis.



Jaeger: Investigating developmental plasticity in Arabidopsis thaliana

for breeding stress-resilient crops. We are studying these processes in two systems: *Arabidopsis thaliana* and the small grass *Brachypodium distachyon*.

We are particularly interested in how plants utilise their developmental plasticity to adapt to their environment. As a model system, we aim to understand how day length influences time to flower, how this environmental signal is sensed, and how this information is integrated direct development. To break down these questions, we are focusing on three research strands: 1) How conserved is the floral induction pathway? 2) How do plants remember temperature stress? And 3) How are growth and development coordinated with environmental signals?

Dr. Devin O'Connor,
Career Development Fellow

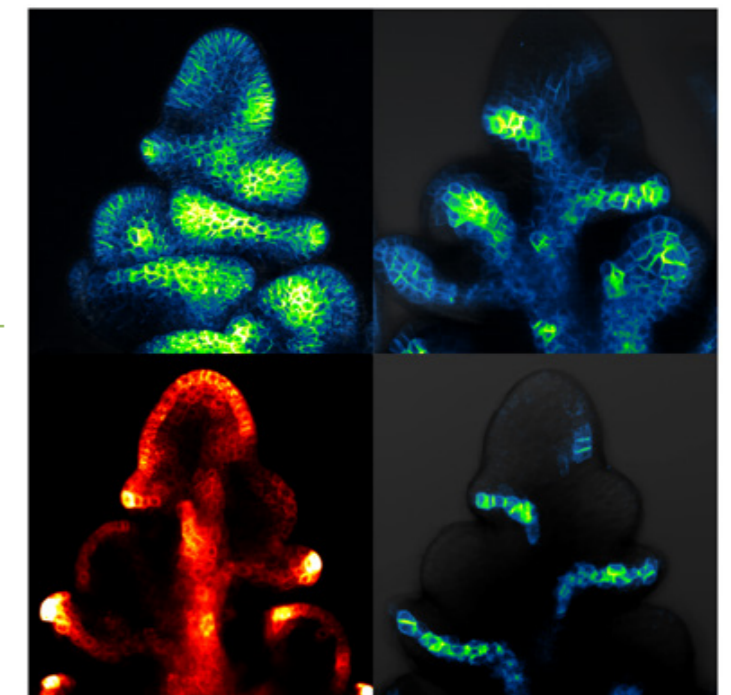
Using *Arabidopsis thaliana* and the grass *Brachypodium distachyon* as model systems, our research centres on three important questions in plant developmental biology: 1) How does auxin transport control the placement of both lateral organs and vasculature? 2) What are the molecular mechanisms that explain the

morphological differences between monocot and dicot plant groups? 3) What are the genes that control the density and plasticity of leaf veins?

At the core of the lab is the discovery that during organ initiation most flowering plant species utilize at least two PIN auxin transport proteins, Sister-of-PIN1 and PIN1, which likely separate the organ initiation and vein patterning roles that are combined in Arabidopsis PIN1. We use comparative genetics and imaging in both these species in an attempt to relate changes in PIN gene family structure, protein expression domain, and protein function in order to determine how PIN polarity is controlled, and subsequently how changes in PIN function may relate to morphological diversity. We are also using natural variation, high-throughput imaging of vein traits, and reverse genetics in *Brachypodium* to identify identify new factors controlling vein patterning in grasses.



Devin O'Connor



O'Connor: Confocal images from Brachypodium spikelet meristems expressing fluorescently-labelled auxin transporters and reporters.

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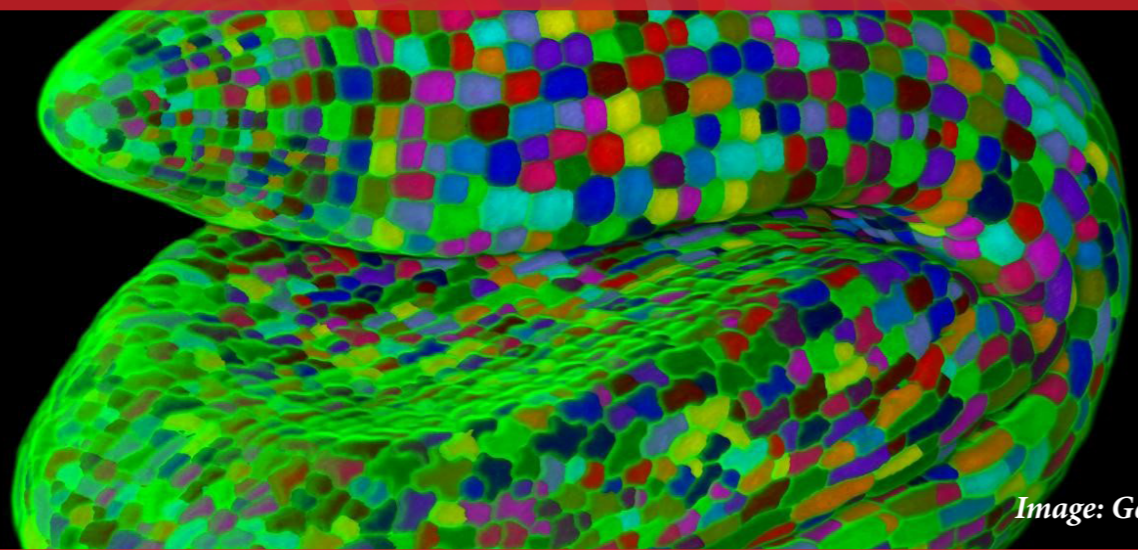


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