GARNish
June 2017: Edition 27

The Wheat Issue

Welcome to the June 2017 Issue of GARNish

Steven Spoel

GARNet Chairman

Welcome to this new issue of GARNish. In previous issues GARNet Coordinator, Geraint Parry, discussed the rise of the EU referendum followed by the Brexit vote and the political as well as economic uncertainty this has created. In writing this first editorial as the new GARNet Chair as well as EU citizen, I feel compelled to continue this storyline as the political uncertainty surrounding the Brexit vote has not yet eased.

At a time when political stability is of utmost importance to secure the best Brexit deal for the British science community, the UK public once again heads to the ballot box but this time for the UK General Elections. Although party campaigns have publically devoted little attention to science, the outcome of the General Elections will certainly influence the way science is debated at the Brexit negotiation table. While this may sound like doom and gloom to many, it should be pointed out that many organisations are fighting our corner to ensure UK science gets the best possible deal. Organisations such as The Royal Society as well as many UK universities that together receive a substantial amount of EU funding have been very vocal about securing the rights of EU students and staff early on in negotiations, and to ensure that the UK maintains access to EU research funds, particularly the Horizon 2020 funding programme. At GARNet we strongly echo these calls for avoiding barriers between the UK and EU that may adversely affect the UK plant sciences community.

In preparation for a post-Brexit Britain, the government has announced a new Industrial Strategy Research Fund (ISCF) to allow the country to take advantage of its strong history in research and innovation. ISCF aims to promote joint research projects between businesses and academic researchers that are led by either industry or universities. Although details will remain scarce until after the General Elections, ISCF is expected to put significant emphasis on challenges in bioscience and biotechnology. Given the strong connections UK plant scientists already enjoy with agribusinesses from around the world, ISCF has the potential to become an important new source of funding and innovation in the plant sciences, perhaps similar to the Global Challenges Research Fund that supports research into challenges faced by developing countries.

In collaboration with BBSRC the GARNet Advisory Committee will be closely following any developments in ISCF and communicate them to UK plant sciences community. At the same time fundamental research in the plant sciences, including Arabidopsis research, remains essential and should not end up at the back of the queue, so new funding plans for a post-Brexit Britain have to take this into account. Importantly, this is also the view of some agribusinesses that already have strong applied R&D activities but lack the capacity to generate fundamental advances currently made by academic plant scientists.

In this edition of GARNish we have a few articles that focus on wheat research that has relevance for Arabidopsis researchers. In addition we also explore the work of the Stockbridge Technology Centre that supports technological developments for the horticultural industry, the Microphenotron that provides a microphenotyping platform for high-throughput screening of chemicals that alter plant growth and development, and the National Institute on Agricultural Botany (NIAB) whose aim is to provide independent knowledge to promote agriculture and horticulture.

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

Contents

Special thanks to: Alison Bentley, Lisa Martin, Jonathan Carruthers, Stephanie Smith, Philippa Borrill, Dheerj Rathore, Phill Davies, Brian Forde, Ian Street, the BBSRC grant holders and the plant scientists at NIAB.

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The GARNet Committee

UK Plant Sciences Federation Update

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During 2016 and 2017, the UKPSF has been consulting with members of the plant science community to produce a Roadmap for plant sciences in the UK. Publication of the Roadmap is planned for October, following extensive external review over the summer. The Roadmap explores the current research landscape, provides recommendations, and outlines priorities in four areas: biodiversity and ecosystem services; plant health and biosecurity; improving crops and agriculture; and biofuels, bioenergy and bioproducts.

The UKPSF will mark the launch of the Roadmap with a one-day meeting in Westminster on 4th October, to which plant scientists and policymakers will be invited to discuss its main findings and themes. The meeting will address goals and challenges for the sector, and provide a forum for delegates to discuss the future of UK plant sciences, and to share their vision of what the sector can achieve.

The UKPSF launched the Plant Health Undergraduate Studentships scheme this year, which aims to address skills shortages in plant health research and provide training opportunities for students. The programme offers paid research projects for four students to address major plant health challenges identified by Defra, who provided funding for the scheme. Following an open call for project proposals, UKPSF advertised the four funded projects to undergraduates. The response from students was overwhelming, with 145 applications received for the four placements, highlighting the demand for internships in plant science. The four successful students will undertake their research projects for 8-10 weeks over summer, and produce reports of their experiences. These projects will investigate genetics of wheat yellow rust disease, transmission of plant viruses, development of diagnostic kits, and the use of citizen surveillance data. The UKPSF hopes to secure funding to offer an expanded programme for next year, and is investigating ways to promote these studentships in collaboration with other organisations offering similar opportunities.

Global Plant Council Update

Lisa Martin,
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Back in October 2015 the GPC held a very successful Stress Resilience Symposium and Discussion Forum in Iguaçu Falls, Brazil. During the symposium, we brought together experts from across the world to discuss current research efforts in stress resilience, showcase new approaches and technologies, and build new networks and collaborations. During the discussion forum the next day, experts in the field worked to develop a consensus view on the strategies needed to develop crops and cropping systems that are better able to deal with fluctuating and stressful environmental conditions.

We're pleased to say that as an output of this meeting, the GPC has now published a series of four commentary papers, and an accompanying editorial, in the open access journal Food and Energy Security (FES).

- The Global Plant Council (Editorial). FES 2017;6(1):3–4.
- The case for evidence-based policy to support stress-resilient cropping systems. FES 2017;6(1):5–11.

Attendees of the Stress resilience Forum. Credit: Jian Bo Shen

- Stress resilience in crop plants: strategic thinking to address local food production problems. FES 2017;6(1):12–18.
- Harnessing diversity from ecosystems to crops to genes. FES 2017;6(1):19–25.
- Integrating islands of knowledge for greater synergy and efficiency in crop research. FES 2017;6(1):26–32.

You can also download the papers from the links at our website: http://www.globalplantcouncil.org/initiatives/stress-resilience.

Talking of meetings, the Global Plant Council (GPC) is currently busy preparing for our next workshop, 'New Breeding Technologies in the Plant Sciences', to be held in association with the Society for Experimental Biology (SEB) and co-organized with our friends at GARNet! This workshop, a satellite event of the SEB's annual main meeting (3–6 July 2017), will take place on the 8–9th July in Gothenburg, Sweden, and is for anyone interested in how technologies such as CRISPR/Cas9 gene editing can successfully be used in plant science research, as well the practical and regulatory issues surrounding their use.

If you're already going to the SEB conference (and I suggest you do as it is always excellent!), why not register to attend our symposium too? You can sign up at http://www.sebiology.org/events/event/new-breeding-technologies-in-the-plant-sciences until 6th June – after that you can register on-site, subject to availability. After the workshop and conference, the GPC will also be holding its Annual General Meeting. This is an opportunity for representatives of our Member Organizations to come together to discuss the GPC's work and future directions.

If you'd like to know more about the GPC and what we do, my colleagues Sarah and Ruth will have a booth at the SEB conference in Gothenburg (I unfortunately won't be able to attend as I will be preparing to bring the first GPC baby into the world!). Please stop by and say hello! In the meantime, don't forget you can interact with us on Facebook (www.facebook.com/GlobalPlantGPC) or Twitter (in English @GlobalPlantGPC and Spanish @GPC_EnEspanol), stay up to date with our monthly e-Bulletin newsletter (http://tinyurl.com/GPCebulletin), or visit our website (www.globalplantcouncil.org) or blog (blog.globalplantcouncil.org).

SEB Cell Satellite meeting: From Proteome to Phenotype: The role of Post Translational Modification in Plant Growth

December 11th-13st 2017,
University of Edinburgh

Organised by Steven Spoel, Cyril Zipfel, Geraint Parry and the SEB

Post-translational protein modifications add a tremendous amount of complexity to cellular proteomes. The large variety of post-translational modifications (PTMs) and their concurrent appearance in proteins dramatically increase the proteome size from mere thousands to the order of millions of possible protein forms. Emerging evidence indicates that in plants crucial regulatory PTMs that control protein function and activity include, amongst others, phosphorylation, ubiquitination, sumoylation, various redox-based modifications, palmitoylation, methylation, riboylation and acetylation. While major advances have already been made particularly in mammalian PTM signalling, the role of PTMs in plant biology is comparatively poorly understood.

Only recently plant scientists have begun to mine the proteome for regulatory PTMs and it has rapidly become clear that PTMs play crucial roles in transforming functional plant proteomes into phenotypes. This symposium is a timely effort to bring together a growing global community of plant scientists that recognize the importance of signalling by PTMs. This meeting will provide an enhanced understanding and appreciation for the role PTMs play in shaping plant phenotypical traits and will be suitable for researchers at any stage in their careers. We particularly welcome early-career PhD students and postdocs, who will benefit most from exposure to the current changing perspective on which factors determine phenotypic traits.

Additionally, PhD students and postdocs can take advantage of a hands-on Plant Proteomics workshop on day 3 of the meeting, which is co-funded by GARNet. This hands-on workshop will be led by Dr Alex Jones (University of Warwick) and will allow researchers to obtain real-time experience in handling proteomics data and utilising systems approaches. Look out for registration details soon!

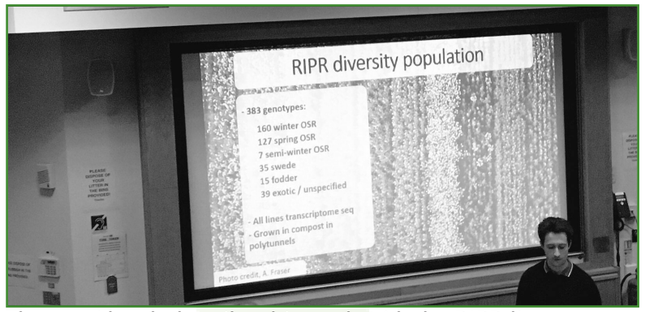
SOCIETY FOR EXPERIMENTAL BIOLOGY

UK Brassica Community Meeting
April 4th-6th 2017
University of Nottingham

Geraint Parry

It was a great pleasure to attend the Annual Meeting of the UK Brassica Research Community (UKBRC). The meeting was hosted by Dr Neil Graham and split into two sections, the first of which allowed for short talks updating on (mostly) PhD student and postdoc projects and the latter providing updates on the resources that are being developed for use by the Brassica research community.

As in 2016 many talks focussed on research that has used the RIPR Oil Seed Rape (OSR) Diversity Set lines that have been developed in Ian Bancroft's lab following support from the BBSRC. Over the course of a few years it is excellent to see the progress of research projects from the early planning stages into the generation of publishable data. Thomas Alcock (University of Nottingham) has used the RIPR lines to search for QTLs that are important in metal tolerance and has found some interesting genes. Similarly Marie Bruser (JIC) used these lines to look at seed pod development and discovered that genes involves in programmed



Thomas Alcock describes his work with the RIPR lines.

cell death participate in this process. Both Thomas and Marie took an experimental approach shared by a number of other speakers, including Richard Broughton (Rothamsted Research) who works on seed phytosterol content, namely that they have discovered interesting Brassica QTLs and then moved their research back into Arabidopsis in order to test the function of orthologs. This strategy again highlights the importance of Arabidopsis for underpinning many aspects of UK crop science.

Rumiana Ray (University of Nottingham) and Graham Teakle (University of Warwick) provided updates on the ICAROS and 'Roots of Decline' projects respectively. ICAROS looks to develop a sustainable protection strategy for OSR against the effects of *Rhizoctonia solani*. Little is known about the overall impact of this pathogenic fungus so part of the project will quantify the UK yield loss and the epidemiology of the disease together with potential future management plans.

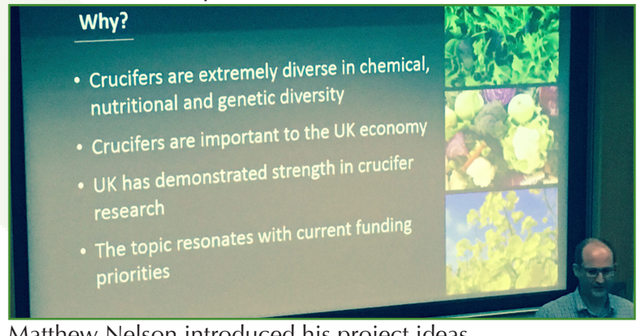
The 'Roots of Decline' project is part of a set of grants funded under the SARISA (Soil and rhizosphere interactions for sustainable agri-ecosystems) scheme. The overall aim of this research project is to determine the effect of the soil microbiome on the growth of OSR. One part of the experimentation involves growth of plants across three UK sites (Wellesbourne, Rothamsted, Harper Adams) in different rotations with wheat. This will hopefully provide important information on how a differing soil microbiome coincides with changing yields of OSR.

After a pleasant lunch kindly funded by the OREGIN grant, Wiktor Jurowski (Earlham Institute) provided an update about the Brassica Information Portal (BIP) and how they are integrating their tools with CyVerse and ElixirUK. They have recently published a pre-print describing the tools available in the BIP and are also planning to hold a Workshop on these tools at the Earlham Institute on June 15th. Please contact Wiktor for details about this meeting.

Lars Ostergaard introduced the very exciting BRAVO project, which is a recently funded BBSRC sLOLA. BRAVO will fund research across seven UK academic sites and importantly provides opportunities for collaboration with members of the agricultural industry. Across five work packages the project aims to understand the gene networks that control flowering time and study how these networks affect all developmental stages, from vegetative growth to seed production. The £4.4million of funding will truly move this area of research forward over the next five years….. look out for its outputs soon.

BRAVO
Brassica Rapeseed And Vegetable Optimisation

The final presentation was provided by Mathew Nelson who is the Research Leader for Crop Plants at Kew Gardens. He hopes to lead a consortium that will investigate the production of novel bioactive compounds that are produced in Brassicas. Look for developments in this area soon!



Matthew Nelson introduced his project ideas

GARNish
Wheat Training Resource

An online training and resource portal for wheat

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A large portion of plant biology is focused on studying model organisms such as A*rabidopsis thaliana* due to a number of reasons: a short life cycle of approximately six weeks, low cultivation requirements, a small genome of ~135 Mb and an easy and effective way of transformation using *Agrobacterium tumefaciens*. As a result, many resources have been generated by the community such as extensive mutant collections and marker lines. This data has been stored and made available in data repositories such as The Arabidopsis Information Resource (TAIR) and Araport, which enabled researchers to quickly and easily search the available data.

From discussions with Arabidopsis researchers, many would like to translate the knowledge they generated into crop plants such as wheat (*Triticum aestivum*). But even simple tasks such as growing and crossing wheat plants is considerably more difficult; learning to perform these tasks requires time and effort while material and methods sections in published articles are often short and cannot substitute teaching aids. This is even more true for more complex topics such as the genomics aspect of wheat and issues surrounding polyploidy

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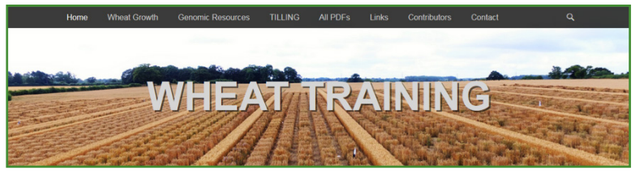
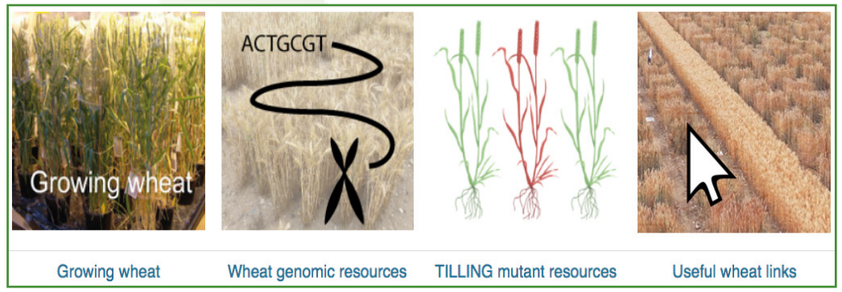


In recent years huge improvements in the tools and resources available to work on wheat have been made. This has made working in wheat a viable and attractive prospect. However, information on these new resources is often difficult to access since it is scattered across multiple sites and requires insider knowledge. With an aim to address this, we developed a wheat training website (http://www.wheat-training.com/) to provide background information and practical resources to help both budding wheat scientists as well as researchers looking to expand their work into wheat.

We were invited to the GARNet2016 meeting in Cardiff to present this website which contains a range of resources including step-by-step protocols, guides to growing wheat and up to date information about genomic tools and mutant collections. The presentations made at GARNet2016 are available at http://www.garnetcommunity.org.uk/reports

The website is divided into the three main sections (i) growing wheat, (ii) genomic resources and (iii) TILLING mutant resources. We indexed the website so that it can be searched for key words and developed each section into pdf documents. This allows users to find the relevant passages that can then be printed off to carry to the lab, glasshouse or field for reference. The website also contains links to other important websites and data repositories for wheat (e.g. CerealsDB, GrainGenes, URGI).



Growing wheat

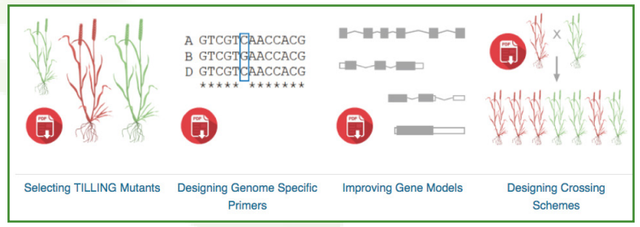
In this section we provide fully illustrated guides to growing wheat covering all stages of the lifespan from germinating the seeds to harvesting and threshing the grain. All relevant steps to cross wheat plants are explained in YouTube videos made by the Germplasm Resources Unit (GRU). This section also contains efficient protocols for everyday wet lab jobs such as extracting DNA. These guides will hopefully provide a starting point for people new to wheat.

Genomic resources

Within the past 24 months, three new wheat genome assemblies have been released. Although it is a welcomed change to now be able to access so much genomic information, knowing which resources to use and when and how to integrate them can be a headache. Therefore, in this section we provide introductory information to the genome assemblies available, their strengths and weaknesses, and where to access the data. We further describe the gene expression atlases (e.g. www.wheat-expression.com) and variation data which are available for wheat. We also provide a detailed guide to help users find the orthologue of an Arabidopsis gene in wheat.

In silico TILLING

Until recently performing functional genomics in wheat was still a pipedream. However within the last six months this has become a reality with a catalogue of exome-sequenced EMS wheat mutants available in both tetraploid and hexaploid wheat. For each of the populations the chance of recovering a deleterious mutation in any gene is over 90 %. In this section we describe how to access and use this resource, as well as strategies for working in tetraploid and hexaploid wheat. We further describe how to design crossing schemes to combine the multiple genome copies of genes in polyploid wheat to create a full knock-out mutant. Furthermore this section shows how to generate genome-specific primers to select mutants using available online tools.



This website is not meant to eliminate the intricacies of working with wheat. However, we do hope that it will provide a useful starting point for researchers interested in working on wheat and provide a community hub to share wheat resources with scientists and breeders already working on this vital crop. We plan to expand the content further based on community feedback, so please do let us know if there are any aspects which you'd like covered so that we can improve the website in future releases.

Introducing the Stockbridge Technology Centre

Dr Phillip Davies

Business manager

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Stockbridge Technology Centre is an independent horticulture research and development company as well as an educational charity based in North Yorkshire. The organisation emerged from the ashes of HRI in 2001 when, with help from donations provided by commercial horticulture businesses, the site was bought from the government. Since that time STC has been working to provide solutions to the numerous challenges growers encounter, from diagnosing pest and disease issues through to the demonstration of novel technologies that can improve production efficiency. Our site consists of 200 acre arable farm where we grow cereal and field vegetable crops for crop variety and pesticide trials for the likes of AHDB, large AgChem and seed companies. We have 3 acres of computer control glasshouses that range in size from 15 to 900 m2. We have a highly experienced farm staff capable of growing any crop to commercial standards and a science team with expertise in plant pathology, entomology, precision farming and photobiology.

As well as providing solutions to the problems that growers encounter on a day-to-day basis, STC is always looking for new technologies that have the potential to drive a step-change in horticultural crop production. LED lighting was one such technology that was identified back in 2010 by Dr Martin McPherson as a technology

Figure 1: One of the twelve light racks with a range of hydroponically grown lettuce varieties.

that has the potential to revolutionise crop production in multi-tiered Urban Farming systems. The result of his interest in this technology was the LED4CROPS research facility that was opened in 2012. It was the high-energy efficiency of LEDs that first caught Martin's attention but it wasn't until we started working in the LED4CROPS facility that the potential of spectral manipulation of crops became fully apparent. As well as building the LED4CROPS facility STC in collaboration with Lancaster University secured a Fellowship funded by a consortium of industry

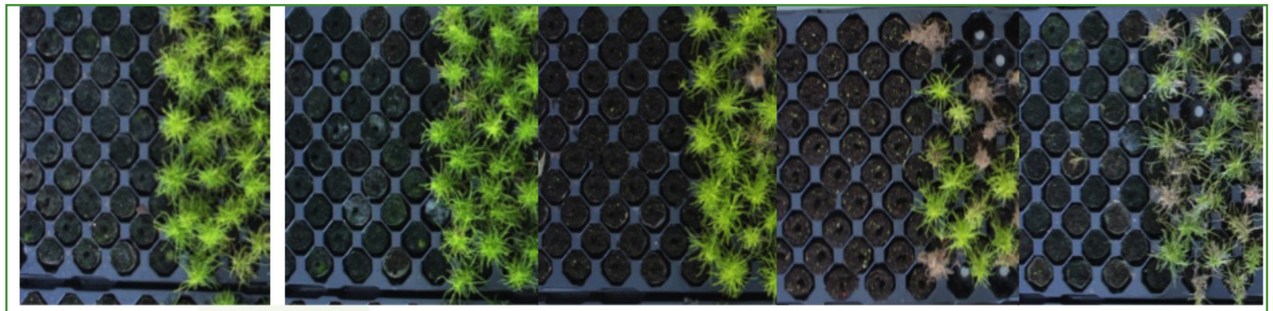

Figure 2: Performance of Santolina 'Lemon Fizz' cuttings exposed to different red:blue light mixtures.

support organisations (Agriculture and Horticulture Development Board, East Malling Trust and the Horticulture Trade Association) to ensure the UK has the expertise to support horticulture businesses make use of advances in LED lighting systems. In late 2012 I filled the post at STC with a remit to drive forwards the R+D of applied LED lighting. It has taken some time but now five years later, businesses are beginning to see the potential of these systems and invest in the technology. We have developed a good understanding of how to grow crops in these environments and how plants respond to changes in light quality. We can now design light treatments to enhance specific crop qualities and help growers make optimal use of this technology.

The LED4CROPS facility is a large controlled environment room containing twelve racks each with four LED lit shelves (Figure 1). Each shelf can be independently controlled allowing plants to be grown under multiple light treatments differing in day length and colour. Four of the racks contain flexible LED light systems that can create any combination of red, blue and far-red light. Under these lights we are able to develop lighting regimes aimed at optimising all stages of crop production.

As part of a three year AHDB funded trial we have used the system to learn which light treatments provide the best conditions for rooting cuttings, which combinations of light produce the greatest crop yields, how to keep plants compact in morphology as well as how to induce flowering in time to hit sales targets. Each of these aspects alone has the potential to improve crop production systems but when combined the potential is staggering.

In our experiments success rates of rooting cuttings varied from greater than 90% rooting down to 20% or less when the colour of the light changes from red to blue (Figure 2). The best

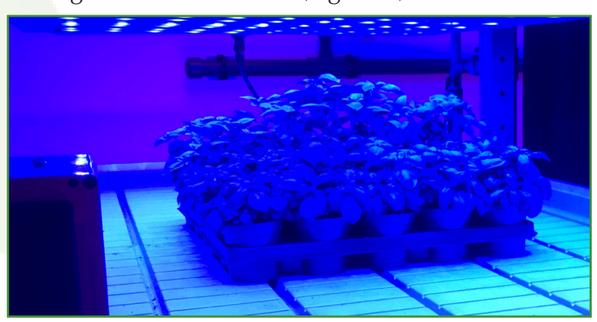

Figure 3: Blue light LEDs

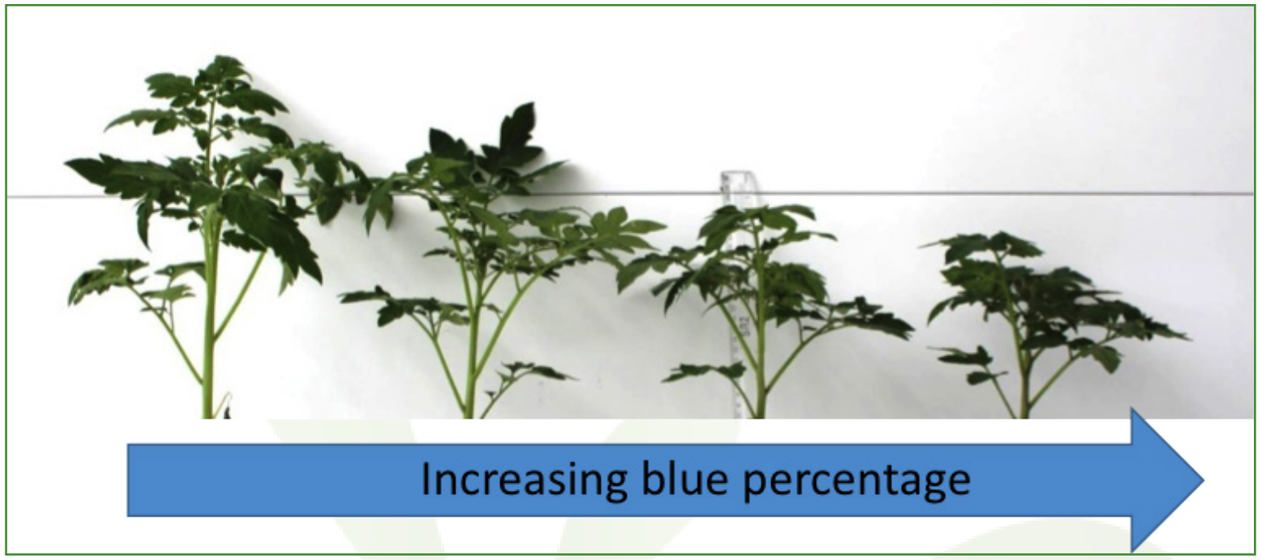

Figure 4: Increasing the blue light proportion causes tomato plants to produce shorter stems and smaller leaves.

light mixtures speed up the process of rooting as well as improving strike rates. Commercial plant propagators that have invested in lighting systems are reporting similarly impressive results. For labour intensive tasks like cutting propagation even small improvements can improve profitability but larger advances can completely change business outlook.

We have also performed trials examining how light quality influences yields and quality of over thirty crop species. Across these species there are some consistent trends in how plants respond to light. Increasing proportions of blue light (up to about 60%) cause plants to produce shorter stems and smaller leaf areas resulting in a compact morphology (Figure 4). This increase in compactness is, however, associated with a drop in biomass accumulation. The greatest biomass is usually achieved under light treatments containing approximately 11% blue light. Increasing the far-red light intensity causes stem elongation but not always increases in total leaf area. In many cases far-red will increase leaf size but this is can be associated with a reduced number of leaves. Far-red light also has a strong influence on flowering and in the case of petunia flowering can occur two to three weeks earlier in the presence of far-red light.

While the light responses of different crops are fairly consistent the real challenge is achieving the correct light environment to produce a plant that meets the desires of the customer. Achieving the greatest biomass may be relevant for some crops but often plants are bought based on their appearance not their mass. To ensure we are able to select a light environment that produces plants with the best qualities we work closely with commercial growers. Our close associations with the horticulture industry gives our research high impact and much of our R&D efforts are focussed on bringing new products and methods to in commercial practice.

Figure 5: The LED4CROPS high wire glasshouse facility. This glasshouse contains four compartments each with a different lighting installation. Photograph provided by Philips

Based on the success of our indoor LED4CROPS facility in 2015 we opened the LED4CROPS high-wire facility (Figure 5) which is a glasshouse designed to compare different lighting systems for production of tomato and other similar crops. We have used this facility to demonstrate the benefits of using LED lighting systems for year round production of tomatoes. Several UK growers have already implemented this technology. Moving forwards this facility will allow a wide range of trials ranging from assessing approaches for energy saving through to assessing how beneficial (bees and biocontrols) and pest insects (white fly and aphids) respond to changes in light quality.

Our lighting research will continue to examine new ways to improve commercial horticulture and we are always looking for new partners to help speed the flow of knowledge from academia to commercial application (in all areas of horticulture and agriculture not just lighting). Currently we a putting our experience in urban farming good use as we plan a new facility aimed at producing a full economic and energy analysis of urban farming systems. Commercial interest in lighting systems is growing and we believe this area of research has a bright future.

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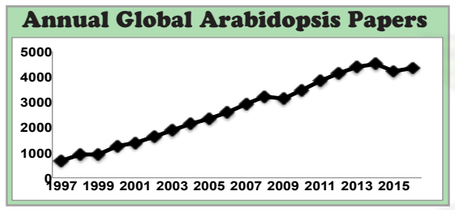
Multinational Arabidopsis Steering Committee

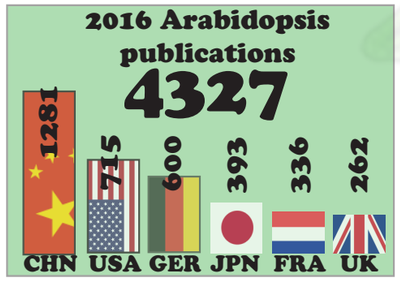
Annual Report 2016/17

Download Full MASC Report: *http://arabidopsisresearch.org/index.php/en/publications*

Country Highlights

> **Argentina**: Legris *et al* (2016) Phytochrome B integrates light and temperature signals in Arabidopsis. Science.
> **Australia**: Several Australian researchers working with Arabidopsis were listed in the Thomson Reuters High Cited Researcher for 2016.
> **Austria**: Nine holders of current ERC Starting and Consolidator grants.
> **Belgium**: New ERC Grants for Bert De Rybel and Daniel Van Damme.
> **Canada**: Continued outstanding development of BAR resource and its integration with Araport.
> **Chile**: Creation of Chilean Society for Plant Biologists.
> **China**: Yao *et al* (2016) A non-canonical hormone receptor for strigolactone. Nature.
> **Czech Republic**: Hosted EPSO plant biology congress "Plant Biology Europe EPSO/FESPB 2016".
> **Denmark**: Olsen *et al* (2016) Mother-plant-mediated pumping of zinc into the developing seed. Nature Plants.
> **Finland**: Hosting 29th ICAR in Turku in June 2018.
> **France**: Continued development of outstanding resources at Saclay Plant Sciences Centre.
> **Germany**: Updated AFGN website: *http://www.dbg-afgn.de/*
> **India**: Challa *et al* (2016). Activation of YUCCA5 by the transcription factor TCP4 integrates developmental and environmental signals to promote hypocotyl elongation in Arabidopsis. The Plant Cell.
> **Ireland**: Fort *et al* (2016) Disaggregating polyploidy, parental genome dosage and hybridity contributions to heterosis in *Arabidopsis thaliana*. New Phytologist.
> **Israel**: Strong support for fundamental research at Israeli Centers of Research Excellence (I-CORE).
> **Italy**: Ezquer *et al* (2016) The Developmental Regulator SEEDSTICK Controls Structural and Mechanical Properties of the Arabidopsis Seed Coat. The Plant Cell.
> **Japan**: 75 scientists selected as Highly Cited Researchers 2016.
> **Netherlands**: Arabidopsis research remains well funded.
> **New Zealand**: Peters *et al* (2017) A conserved cis-regulatory module determines germline fate through activation of the transcription factor DUO1 promoter. Plant Physiology.
> **Spain**: Martin *et al* (2016) Phytochrome and retrograde signalling pathways converge to antagonistically regulate a light-induced transcriptional network. Nature Communications.
> **South Korea**: Welcomed 1000 delegates from 29 countries to ICAR2016 in Gjeongju.
> **Sweden**: Porco *et al* (2016) Dioxygenase-encoding AtDAO1 gene controls IAA oxidation and homeostasis in Arabidopsis. PNAS.
> **Switzerland**: Doblas *et al* (2017) Root diffusion barrier control by a vasculature-derived peptide binding to the SGN3 receptor. Science.
> **United Kingdon**: BBSRC funded GARNet continues to support all aspects of Arabidopsis research.
> **United States**: Hosting the 2017 ICAR conference.





New Arabidopsis Grants

By our calculations of the BBSRC responsive mode grants awarded in 2016, there were less than 20 funded grants that included any amount of Arabidopsis research. This is lower than in previous years so we will continue to monitor this to ascertain whether this a trend or a blip. If the former is true then GARNet will continue to discuss this issue with BBSRC to ensure that basic plant science research is supported, knowing as we do that it importantly underpins research in other plant species.

Perception and integration of nutritional signals in plant root systems: Solving the mystery of K-Fe-P interactions.

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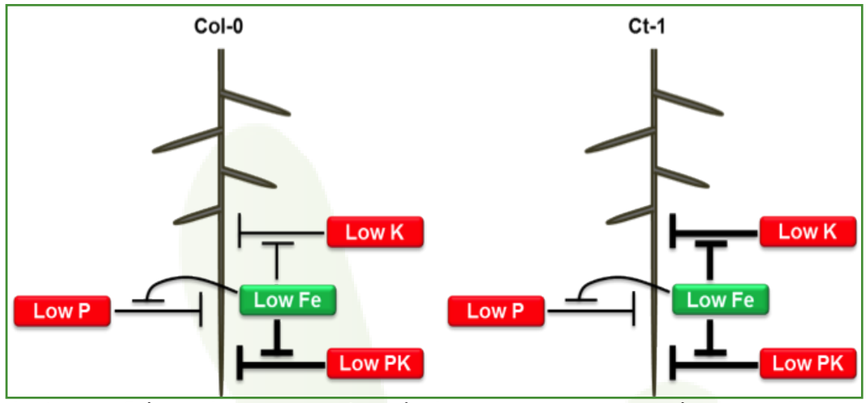
Plant roots are crucial for efficient uptake of mineral nutrients and water. During their life, roots are exposed to fluctuating nutrient availability in soil. Roots need to perceive these nutritional signals and integrate them into their physiological and developmental programs to adapt the root shape and transport activity, thereby optimizing foraging and uptake capacity. How the root architecture changes in response to edaphic signals depends both on the environment and on the genetic makeup of the plant. For instance, primary root length of Arabidopsis thaliana is strongly inhibited by phosphorous (P), potassium (K), or iron (Fe) deficiency, but not by sulfur or zinc deficiency (Gruber *et al.*, 2013, Plant Physiol.). In terms of genetic variation, it is well documented that some Arabidopsis accessions such as Shahdara (Sha) are very sensitive to P limitation while others such as Bayreuth (Bay-0) show little sensitivity (Svistoonoff *et al.*, 2007, Nat. Genet.). In our lab we observed a remarkable variation between Arabidopsis accessions for RSA response to K limitation; Columbia (Col-0) maintains growth of the primary root, but halts lateral root extension, while Catania (Ct-1) stops main root growth, but extends lateral roots (Kellermeier *et al.*, 2013, Plant Physiol.). And yet the two accessions look very similar when grown with sufficient K.

Surprisingly, the low-K induced primary root growth restriction can be reverted by lowering Fe in the growth medium in both Col-0 and Ct-1. Iron availability is well known to be important for the primary root response of Arabidopsis to low P, and the major genetic determinants underpinning this mechanism have been identified (Svistoonoff *et al.*, 2007, Nat. Genet. Müller *et al.*, 2015, Dev. Cell. Mora-Macías *et al.*, 2017, PNAS). Interestingly, despite the striking differences in their response to low K, Ct-1 and Col-0 do not differ in their response to low-P, both showing similar primary root inhibition. This suggests the existence of yet to be identified mechanisms controlling the low-K response and the role of Fe in this response. Similar to P limitation, K deficiency leads to hyper-accumulation of iron in the root apical meristem which is more pronounced in Ct-1 than Col-0. However, in contrast to P, K does not form a complex with iron, and therefore we have to look for different modes of interaction. One possibility is that K modulates Fe transport within and between cells because it makes a major contribution to the driving force (membrane potential) and to the electric counter-balance of ion movement.

To add to the complexity we found that Col-0 and Ct-1 also differ for root growth response to combined P and K deficiency. Low K and low P additively inhibit root growth in Col-0 while low K dominates over low-P in Ct-1. These data reveal a complex interaction between K, P, and Fe in terms of their effects on root growth and development, but they also offer hope that the complexity can be unravelled by exploiting the natural genetic variation.



Amtmann: Schematic representation of primary root responses to low potassium, phosphate, and iron applied as single and combined deficiencies in Col-0 and Ct-1 accessions of *Arabidopsis thaliana*. Thin and thick bars respectively represent weaker and stronger inhibitory effects of nutrient deficiencies on primary root length.

In our BBSRC-funded project (BB/N018508/1) we will combine electrophysiological methods and confocal microscopy with molecular quantitative genetics and automated root phenotyping to address the following questions: How is K perceived by root cells and what is the link to iron? How do roots sense and transduce signals of combined K, P, and Fe deficiency? How are developmental responses of the main root coordinated with those of the lateral roots? How do different root architectures impact on nutrient uptake and on final nutrient contents in the shoots? The results from this study can be expected to identify major genetic factors underpinning the crosstalk of signalling pathways that control root system adaptive response to mineral nutrient supply. In their natural habitat, plants are exposed to a varying ratio of different mineral nutrients over the season. Evolution has responsed to this environmental challenge with a physiological, developmental and genetic plasticity that provides us now with an ample resource for crop improvement strategies aimed at enhancing a plant's capacity to resist multiple environmental stresses. The planned research is therefore not only important from a fundamental scientific point of view, but may also open new avenues towards food security.

Controlling dynamic S-acylation in plants

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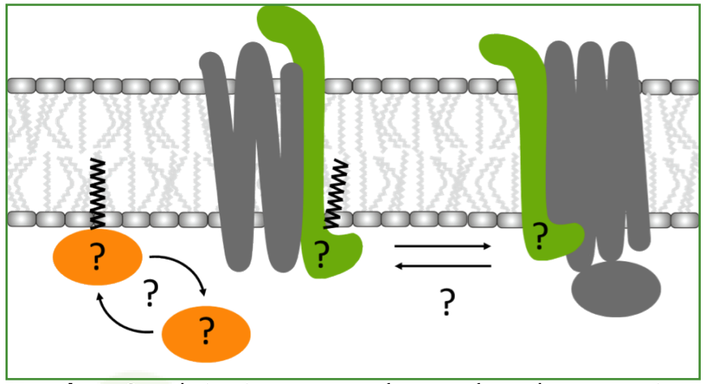
Membranes provide the barrier between the plant cell and its environment as well as compartmentalising diverse and frequently incompatible cellular functions and activities. Regulating membrane structure and formation, as well as the transport of solutes, protein, metabolites and signals across them, is essential for maintaining a properly functioning cell. Much of this regulation is achieved through the action of proteins and the modulation of their activity by post-translational modifications.

S-acylation is a lipid based post-translational modification primarily thought to alter how a protein interacts with its membrane environment. We recently demonstrated that S-acylation is a common feature of integral and peripheral membrane plant proteins, with our best estimates suggesting that S-acylation is

found on >1/3 of the membrane proteome. Despite this knowledge we know very little about how S-acylation regulates or affects protein function. S-acylation is known to be reversible and dynamic, and has been proposed to act as a regulatory mechanism in a similar way to phosphorylation or ubiquitination but, as yet, we know the identity of very few proteins in plants regulated in this manner. The mechanisms surrounding changes in S-acylation state are also unclear; while we know the identity of S-acylating enzymes we have no information on how de-S-acylation is achieved in plants.

We recently identified a number of compounds able to prevent de-S-acylation in plants, and treatment of plants with these inhibitors led to a range of diverse and pleiotropic phenotypes. This underlines the importance of correct regulation of S-acylation in normal plant cellular function. The aim of the proposed work is to use these inhibitors as chemical tools to pull out de-S-acylating enzymes in an activity based protein profiling approach and identify them by mass spectrometry. Identified activities will then be characterised biochemically and genetically to determine which activities represent bona-fide de-S-acylating enzymes.

However not all S-acylated proteins undergo changes in S-acylation state as part of their cellular function. To differentiate those that do change S-acylation state from those that do not we will use our inhibitors of S-acylation and de-S-acylation in conjunction with various stimuli. This will enable us to identify proteins from plants that undergo cycles or changes of S-acylation or de-S-acylation as part of their normal function. Proteins identified in this manner will provide us with test cases to gain a better understanding of how the S-acylation states of proteins change and what the effects of S-acylation on proteins are. With this knowledge we will be in a better position to understand if and how we can manipulate one of the fundamental mechanisms involved in the regulation of diverse cellular processes in plants for our benefit.



Hemsley: : S-acylation is a common feature of membrane proteins and can be added or removed from proteins in a regulated manner to control various processes in the cell. How this happens and which proteins are regulated by changes in S-acylation is currently unknown. The work proposed will identify the enzymes that remove S-acyl groups and identify which proteins undergo changes in S-acylation (orange and green) as part of their function

Uncovering the mechanism(s) controlling crop growth promotion by phosphite

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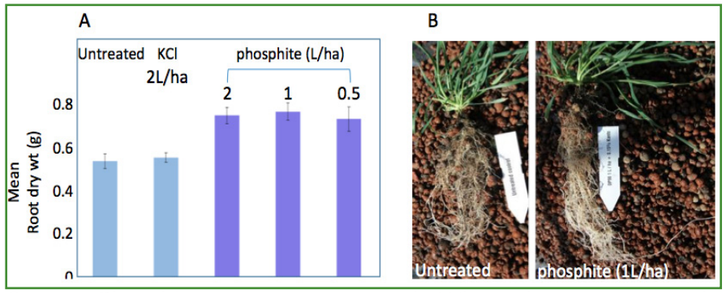
Global food security is one of the biggest challenges facing world agriculture. Significant improvements in crop yields are urgently required to meet the 50% increase in world population by 2050. There have been several studies and reports that suggest that improvement in root architecture can have profound impact in improving crop productivity and resource use efficiency.

Phosphite represents a reduced form of phosphate that belongs to a new class of crop growth promoting chemicals termed biostimulants. Foliar application of phosphite enhances root growth and development in a range of plant species, typically increasing biomass by 30%.



Swarup: Phosphite promotes root growth. Wheat plants were treated with potassium phosphite based formulation at stage GS12 and harvested at GS23 and root dry weight measured. Root dry weight only increased upon phosphite (not K) treatment.

Phosphite cannot be converted to phosphate, so does not enhance plant growth via a nutritional mechanism. This BBSRC LINK proposal with six industrial partners aims to identify the mechanism(s) through which phosphites promote root growth. We will employ a multidisciplinary approach involving a combination of various 'omic', cell biology, plant physiology and CT imaging techniques. RNAseq and hormone profiling have identified several promising mechanisms that will be characterised during this project. In parallel, the six industrial partners and ourselves will explore the physiological basis for phosphite's ability to improve resource use efficiency and crop establishment. The knowledge gained from this study will provide information about the key genes and processes controlling root architecture. Also by optimising doses, timing of application and treatments this project will provide a clear framework for phosphite treatment in a number of crops. With crops yielding better returns, this research is likely to have a direct impact on farm income leading to improved nutritional, financial and social stability.

This work is highly collaborative, involving a multidisciplinary team of Nottingham scientists with specialist skills in these areas together with our six industrial partners. Four of our partners are UK based (Brian Lewis Agriculture Ltd T/A Intracrop, Headland Amenity Ltd, OMEX Agriculture Ltd and Verdesian Life Sciences). The other two are European companies Biolchim S.p.A (Italy) and Trade Corporation International SAU (Spain). Our industrial partners have excellent track record in research, development and marketing of a range of biostimulant products including phosphite. They will undertake a series of studies on biostimulant properties of phosphites in glasshouse and field conditions and thus complement the physiological studies done under more controlled conditions in Nottingham. They will test different formulations, doses and their effect in a range of crops and in different agro-climatic conditions in several different countries including UK, Spain, Italy, Germany, Czech Republic, Finland, Canada and Brazil.

By having a better understanding of the molecular and physiological role of phosphite in improving root architecture, this research proposal is likely to have a direct impact in improving resource use efficiency and plant fitness in a number of commercially important horticultural and cereal crops. This allows us to enter a new area of precision farming where traits may be deliberately manipulated via application of non-harmful chemicals.

This work is supported by a set of Industrial Partners: Biolchim S.p.A, Brian Lewis Agriculture Ltd, T/A Intracrop, Headland Amenity Ltd, OMEX Agriculture Ltd, Trade Corporation International SAU, Verdesian Life Science.

Dynamic re-programming of the cold transcriptome in Arabidopsis

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Allan James, Hugh Nimmo (University of Glasgow)

Katherine Denby (University of York),

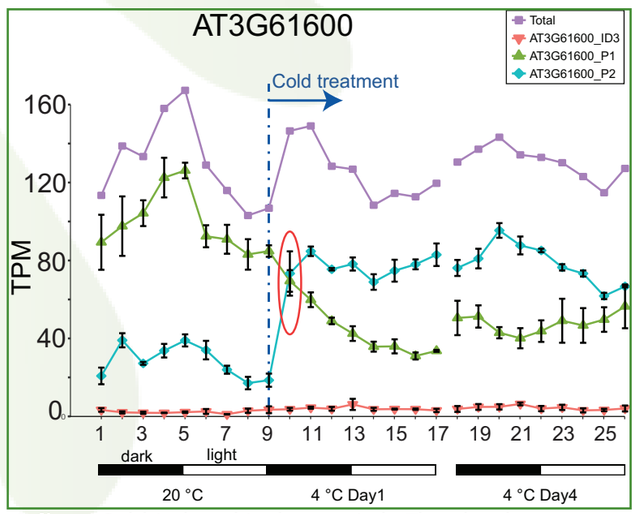
Runxuan Zhang (The James Hutton Institute)

Plants respond to changes in environmental conditions by drastically reprogramming their gene expression. The groups of John Brown, Hugh Nimmo and Runxuan Zhang (University of Dundee, the University of Glasgow and the James Hutton Institute) with funding from BBSRC have been studying how plants (*Arabidopsis thaliana*) respond to cold stress. They have developed a new pipeline to analyse ultra-deep RNA-sequencing data of a high resolution time-course of plants transferred from 20°C to 4°C. This allowed detailed expression analysis at the individual gene/transcript level and demonstrated the major contribution of alternative splicing (AS) to the cold response.

AS generates more than one transcript from a gene and can regulate expression levels or gene function. A substantial proportion of transcriptome reprogramming involves AS (~25-30% of genes) and over 2,000 novel cold response genes have been identified, two-thirds of which are only regulated at the level of AS. In particular, there is a rapid and sensitive AS response which occurs early as the temperature decreases with some AS preceeding the major transcriptional response. In addition, many AS changes persist through the cold treatment and are potentially adaptive, contributing to acclimation and freezing tolerance. A knock-out mutation of a gene with a rapid, significant and adaptive change in AS is freezing sensitive, linking AS to the cold response.

Finally, the high resolution transcript-specific expression profiles generated show changes in rhythmic expression/AS of genes and transcripts after transfer to the cold, reinforcing the integral involvement of the circadian clock and/or photoperiodicity in the cold response.

The new 3-year BBSRC grant to the above groups and that of Katherine Denby (University of York) will analyse in more detail the speed of change in AS relative to the transcriptional response and factors involved in regulation of the AS response. Cold-induced changes in rhythmic expression suggest coupling or de-coupling from the circadian clock and/or photoperiodic responses and such genes will be examined to address the underlying mechanisms of control. A major goal will be to use the transcriptome expression data to build both gene and splicing networks and then test splicing factors that are predicted to have a major role in driving changes in the cold transcriptome. The research will greatly increase our knowledge of the complexity of how plants alter their gene expression to survive low and freezing temperatures and may identify key regulatory genes which may be exploited in crop improvement.



Calixto: Cold-induced isoform switch. The plot shows transcript-specific and total gene expression data (colour-coded) across 26 time-points (3-h resolution) of Arabidopsis 5-week old rosettes transferred from 20°C to 4°C. TPM, transcripts per million; red oval, isoform switch.

More importantly, the research groups involved demonstrate how very different expertise and knowledge can be brought together to develop new approaches and techniques to exploit the power of front line technologies and deliver new discoveries. We anticipate that the contribution of AS to the dynamic changes in the transcriptome seen in the cold response will be occurring in other responses to environmental conditions, pests and pathogens, and during development. As such, AS is a relevant and essential aspect of addressing biological processes in any plant or crop species.

S-acylation of the cellulose synthase complex

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Cellulose is an important component of lignocellulosic biomass which can be converted into biofuels and other economically important chemicals. Consequently, there is widespread interest in understanding how plants make cellulose. Cellulose in higher plants is synthesized by a large plasma membrane localized protein complex known as the cellulose synthase complex (CSC). The known components of the CSC include the CESAs, KORRIGAN, CC, CSI and CMU proteins. The CSC moves in the plasma membrane along the microtubule tracks [1] synthesizing cellulose. However, it is far from clear that how the complex get assembled and trafficked to the membrane and why its movement through the plane of plasma membrane is not hindered by other plasma membrane proteins.

We have recently shown that the CSC is extensively modified by S-acylation of the CESA proteins [2]. S-acylation is a post-translational protein modification where a fatty acid, usually palmitate or stearate is added to a cysteine residue in the protein. We have shown by mutagenesis that a single CESA protein contains 6 S-acyl modifications and since the CSC is likely to be at least an 18-mer we calculate that a single CSC is likely to contain more than 100 acyl groups. This represents S-acylation on an unprecedented scale that will dramatically increase the hydrophobicity of the complex essentially locking the CSC into these special plasma membrane domains during cellulose synthesis. Other components of the CSC machinery like Korrigan and tubulin are also modified by S-acylation [3-5].

These discoveries have allowed us to generate several testable hypotheses regarding how acyl modification and membrane partitioning can contribute to co-localisation of all proteins involved in cellulose synthesis and to the alignments of cellulose deposition with the underlying cortical microtubules. It is possible that S-acylation of the CSC may drive the formation of plasma membrane microdomains that allow unimpeded movement of the CSC through the plasma membrane. We will employ a suite of molecular genetic and biochemical approaches to understand if and how various components of the CSC machinery are S-acylated and whether S-acylation is responsible for maintenance of the structure and function of the CSC. We will also investigate whether the S-acylation of the CSC contributes to all the difficulties we have had in purifying an intact complex and to what extent this modification is important for driving membrane partitioning and co-localisation of the CSC with cortical microtubules.

1- Paredez *et al* (2006). Visualization of cellulose synthase demonstrates functional association with microtubules. Science 312, 1491-1495.
2- Kumar, M *et al*(2016). S-Acylation of the cellulose synthase complex is essential for its plasma membrane localization. Science 353, 166-169.
3- Hemsley, P.A *et al* (2008). Assaying protein palmitoylation in plants. Plant Methods 4, 2.
4- Hemsley, P.A *et al* (2013). A proteomic approach identifies many novel palmitoylated proteins in Arabidopsis. New Phytol. 197, 805-814.
5- Srivastava, V *et al* (2016). Proteomic Analysis of a Poplar Cell Suspension Culture Suggests a Major Role of Protein S-Acylation in Diverse Cellular Processes. Front. Plant. Sci. 7.

AutoRoot: the challenge of fully-automated phenotyping

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Robotic imaging systems such as Lancaster University's Microphenotron are able to capture large numbers of images at regular intervals, enabling the growth dynamics of thousands of seedlings to be monitored as they respond to different chemical treatments or nutritional conditions1. Whilst such robotic setups neatly solve the repeatability problem when capturing large sets of image data, we are left with sizeable quantities of image data which then require analysis.

To date, much image analysis to support plant science has been semi-automatic. This is not true of all available software; certainly, some measures can be made fully automatically, such as measuring the area occupied by green pixels in an image, or length measures of simple structures. But for more complex features, software has been developed more in a supportive role, assisting a biologist expert to annotate or measure images, rather than working through the images without intervention. For many tasks this is entirely appropriate: the user can confirm the results, as they are manually examining every image, and can correct the software to provide high accuracy results. The downside of this approach, though, is that it is still labour intensive (though less so than entirely manual operations), and still only as accurate as the person doing the annotations is careful and attentive. As datasets become larger, the need for fully automated analysis rises.

Full automation comes with a number of challenges. First, no automated method will work perfectly all of the time. With no user in the loop, we must recognise this in the software design. Second, some traits are beyond the reach of current state of the art systems. Precisely measuring total length of a root system, when roots can overlap each other (such as the wells in Figure 1), is a challenge for a human expert; software will struggle to provide an automated result, though, again it can provide semi-automated assistance.

As a solution to these challenges, it is possible to measure simpler traits, which still capture the essence of the phenotype, but which are more suited to automated image capture and analysis. Whilst replicating the measures used by biologists can at times be a challenge, automated measuring can allow for a new, more statistically-derived set of measures.

Whilst a person may be able to use prior knowledge to unpick the separate roots in a complex architecture to provide a detailed segmentation, instead automated software can provide, for example, a bounding box which covers 95% of the root system. Such a measure is not sensitive to occasional miss-identified roots, and does not require detailed detangling of a complete architecture. But still, it provides a number of useful measures to help identify phenotypes, based on spread, depth, etc. Whilst

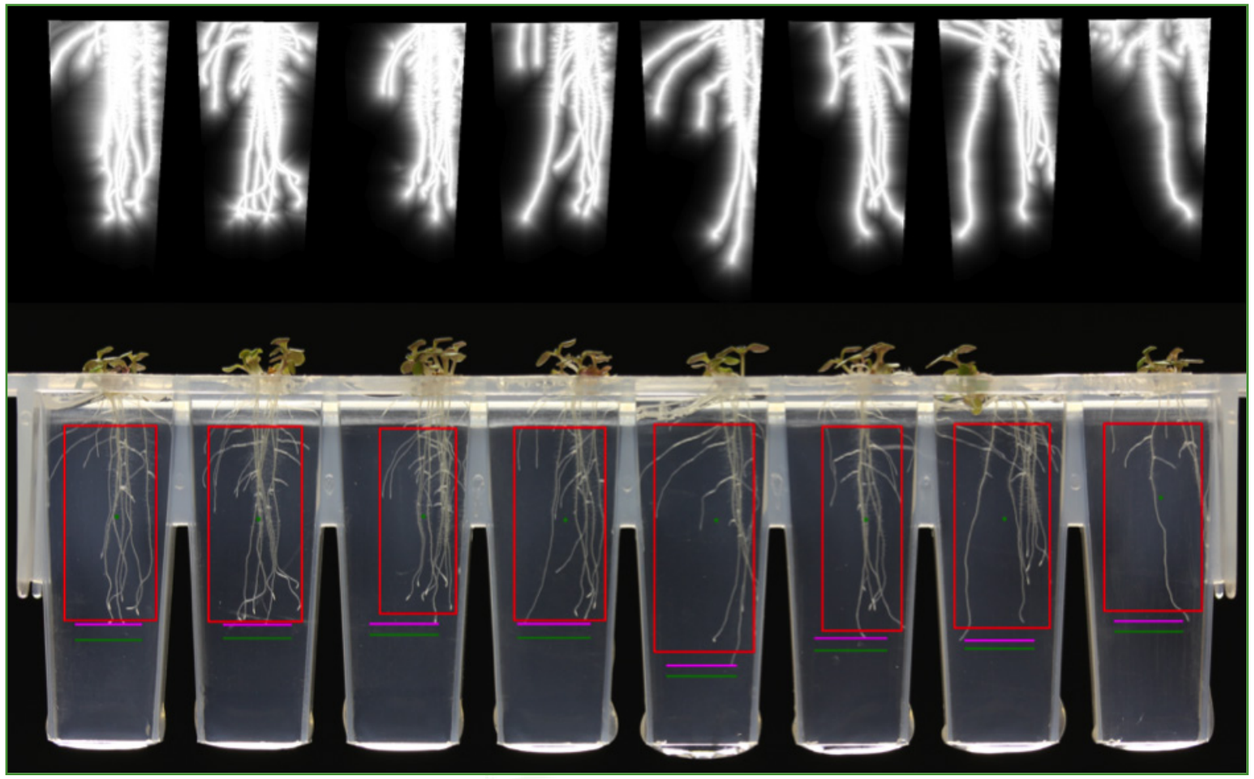

Figure 1. Top row: identifying the likelihood of root material located within the image. Bottom row: Visualisations of various proxy traits, such as centre of mass, 95% bounding box, depth of root system at various confidence levels. See paper for more details (2)

not directly measuring traditional features, they can be used as a proxy to traditional measures such as root depth etc., or they can be used directly to discriminate phenotypes.

This is the approach taken in AutoRoot (2), software designed specifically to support the analysis of Microphenotron images. Rather than firmly labelling a pixel as containing root material, we instead assign a likelihood that it is root. Using this, we calculate various proxy traits fully automatically (See Figure 1). By removing the need to certainly and perfectly segment the root system before measurements are made, we provide flexibility to the software. As we move towards phenotyping systems which produce larger and more numerous image datasets, approaches such as this become a practical solution to the analysis challenge.

1. Burrell, T. *et al*. The Microphenotron: a robotic miniaturized plant phenotyping platform with diverse applications in chemical biology. Plant Methods 13, 10 (2017).

2. Pound, M. P., Fozard, S., Torres Torres, M., Forde, B. G. & French, A. P. AutoRoot: open-source software employing a novel image analysis approach to support fully-automated plant phenotyping. Plant Methods 13, 12 (2017).

Monogram 2017

4th-6th April, University of Bristol

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Sometimes occupying the space between two disciplines can be a lonely place, with each side seemingly so near - yet in practice, so far. So thanks are due to GARNet, who are helping me and others to walk the bridge between the familiar ground of Arabidopsis and the cereal crops which are the primary focus of the Monogram conference.

I am a Postdoctoral Research Associate at the Sainsbury Lab, University of Cambridge. This is my first postdoc, which I've been working on for nine months. Prior to that, I did my PhD in Nottingham looking at root architecture in *Arabidopsis thaliana*. So why, you might ask, am I now doing a project concerning nitrate use efficiency and shoot branching in the model grass *Brachypodium distachyon*?

Making the jump from working with dicots to monocot grasses was a learning curve; there are whole new anatomies, physiologies, developmental differences, layers of complexities, differences in timescales and general little quirks that one does not appreciate when immersed solely in the Arabidopsis world. But like a recent born-again convert who instantly seeks to convert others, I was impressed by the many benefits of working with Brachypodium, and hoped to convince some cereal skeptics that Brachypodium really can be a useful model for them - and conversely, some Arabidopsis researchers that Brachypodium really is a great candidate for bridging the gap in translation between Arabidopsis and cereals. What better pulpit for me to preach from than Monogram?

The Wills Memorial Building
Photo: Stephanie Smith

The venue for Monogram was the stunning Wills Memorial Building, an imposing piece of Gothic architecture standing 65 metres tall (which was a real stroke of luck for a Bristol novice like me with a questionable sense of direction – there were no problems finding the venue!). The Monogram conference itself was divided into six broad sessions: Quality & Nutrition, Genomic Technology, New Projects and Areas, Phenotyping Technology and Applications, Crop-pathogen interactions, and the GARNet session: From Arabidopsis to Cereals and Back Again. There was also an optional Cereal Bioinformatics workshop chaired by Cristobal Uauy. The delegates came from far and wide across Europe and beyond: the usual suspects of Rothamsted, John Innes Centre and NIAB were joined by speakers and attendees from Norway, Ireland, Germany and even Australia, the USA and Mexico.

After the welcome address by Prof. Keith Edwards we kicked off the 'Quality & Nutrition' session with a well-rounded talk from keynote speaker Odd-Arne Olsen demonstrating how molecular biology, cell biology, genomics and transcriptomics have shaped our understanding of the cereal endosperm. The presentation neatly showed how different cell types within the endosperm (endosperm, aleurone and transfer cells) are the result of different developmental programs: endosperm is the default developmental program and is 'locked in' either immediately before or at point of fertilisation, with sucrose providing a crucial developmental trigger. Maternal signalling is required to specify the transfer cell fate, whilst aleurone cell fate is specified by surface cell position and the calpain protease DEK1.

Six more talks followed, featuring research in combatting pre-harvest sprouting (Oluwaseyi Shorinola), trade-offs between grain yield and grain quantity in wheat senescence (Sophie Harrington) and some very, very important research in how we can improve whiskey and beer production and quality (reducing viscosity of grain extract; Till Pellny and improving malting quality of UK winter barley – Bill Thomas).

After some surprisingly entertaining flash presentations and looking around the many posters (all of very good quality) it was time to sample some local ciders and cheeses (well, when in Rome…) - and to take a walk up to the top of the Wills Memorial Tower. After what felt like endless stairs (which went some way to alleviate the cheese-calorie guilt) we were treated to stunning views of the sun setting over Bristol.

Fast forward to day two, and it was time for the GARNet session. Thanks to the GARNet travel award I was able to talk about my work with CINTRIN, the Cambridge-India Network for Translational Research in Nitrogen. CINTRIN's aim is to reduce the dual issues of over-application of nitrate fertiliser in India (which currently is causing extensive environmental damage), whilst still meeting pressure to keep yield high enough to feed India's 1.2bn and growing population. Thus, there are six work packages ranging from the fundamental research I am involved with with a developmental focus (work package 1) - going through each stage of the translational pipeline to genomics led pre-breeding (work package 3) and applied agronomic management strategies (work package 6). My research follows on from observations previously made in Arabidopsis - different accessions respond in two contrasting ways to nitrate limitation: a 'live fast, die young' strategy of prioritising reproduction, or a 'wait in hope strategy' of remaining in a vegetative state for longer, gambling on an increase in

The author delivering her talk at Monogram17 with GARNet committee member Zoe Wilson as session chair. Photo: Alison Bentley

nitrate availability and capitalising on this more effectively if it does happen. I was glad that the pictures of James Dean vs George Clooney to illustrate these contrasting strategies raised a few laughs!

I went on to explain how I'm trying to establish the basis of this nitrate sensitivity and corresponding developmental response in Brachypodium with various genetic methods (and of course, had to include a slide explaining the benefits of my new favourite model).
Luckily I was not alone in my optimism about research in models – other talks in this session included some fascinating work from Ian Henderson about using Arabidopsis and wheat to study the relationship between chromatin, recombination and transposable elements – the latter of which are in no short supply in vwheat! Candida Nibau also used Arabidopsis as a model for investigating recombination and chromosome pairing - looking at the function of Ph1-like kinases in these processes. Syabira Yusoff joined me in extolling the virtues of Brachypodium as a model for her work investigating grain morphology and evolution, whilst Alastair Hetherington gave an enlightening talk on how transpiration and seed iron and zinc concentration are related – and how he is trying to manipulate the signalling pathways involved in order to combat the surprisingly common nutritional deficiencies of these nutrients in humans.

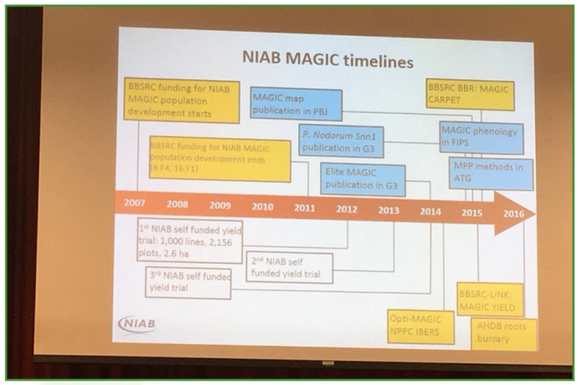
Several more presentations followed, including an enjoyable talk from MECEA PhD student award winner Jemima Brinton about her work in grain size and grain yield relationships in wheat, and keynote speaker for the genomics session, Susanne Dreisigacker, showing how CIMMYT are using genetic marker collections and other genomic technologies to speed their genomics-assisted breeding programme for wheat. In the 'New Projects and Areas' session, invited speaker Keith Edwards gave a talk sure to be of interest to many – his progress in effectively using the CRISPR/Cas9 gene editing system in the notoriously recalcitrant hexaploid wheat, using modified wheat codon-optimised constructs.

MECEA award winner Jemima Brinton with her supervisor Cristobal Uauy. Photo: Alison Bentley

I cannot write this review without including the highlight of the conference – the conference meal aboard the SS Great Britain, Isambard Kingdom Brunel's triumphant ship which first sailed in 1845. The food was divine and the wine flowing…a little too much perhaps! The next Monogram – to be held at the John Innes Centre in Norwich – certainly will be challenged to beat that venue!

On the final day of the conference, we opened with the Phenotyping session – with keynote speaker Tobias Wurschum showing a new strategy to integrate phenotyping with genomics in cereal breeding, using the precision phenotyping platform 'BreedVision' in concert with genetic mapping in a Triticale model. Other interesting advances in phenotyping techniques followed, including biomedical approaches such as X-ray CT to study grain morphology (John Doonan) and how the recombinant inbred 'MAGIC' population developed by NIAB has been a useful resource in linking phenotypical traits with genotype (Anyela Camargo, Yeorgia Argirou).

After heads were soothed with plenty of fluids and strong coffee over lunch, we came to the final session: crop-pathogen interactions. This was the session topic I was least acquainted with, so invited speaker Darren Soanes did a great job in delivering an accessible and illuminating talk about the spread of wheat blast fungus from South America to Bangladesh - and how comparative genomics can be used to track this pathogen in relation to viable host populations, facilitating attempts to stem the devastating yield losses caused. Other highlights included a genome-wide association study of 1000 spring wheat accessions to find sources of resistance to wheat stripe rust (Josh Hegarty) and some very comprehensive work by Emma Wallington concerning how to engineer wheat for resistance to take-all fungus – an especially challenging issue as neither wheat nor any of its relatives have resistance to this fungus, so the answer has to come from gene transfer from oat, and the antimicrobial avenacin A-1 it produces.



A timeline of the NIAB Magic lines
Photo: Geraint Parry

The four-hour drive back home gave me plenty of time to digest all I had heard and seen at Monogram. I came away from the conference feeling inspired and motivated, and it also renewed my respect for the challenges of working with cereals - especially wheat. I am enthused about the new contacts I've made and the interesting conversations I've had – which even persuaded me to finally join twitter! I was also heartened by the thought that despite past pessimism for translational approaches, the mood seems to be slowly shifting, and there are really promising examples of work in this area. Importantly, examples of two-way approaches from either side of the divide also seem to be becoming more common.

I am grateful to GARNet for awarding me the travel award that allowed my attendance at this conference, and for all they do to promote translational work and relationships generally.

Apologies to all the delegates whose work I was not able to highlight for space reasons.

Fascination of Plants Day: Botany Live

#BotanyLive

Geraint Parry

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In 2012 the European Plant Science Organisation (EPSO) started global 'Fascination of Plants Day', an event that aimed to raise the profile of plant science and plant scientists around the world. Since two initial consecutive years this has now switched to a biannual event that has been fully embraced by people who work in on all aspects of plant science. Details of this can be found at http://www.plantday.org. In the UK the events were coordinated by Dr Dario Breitel at the John Innes Centre and featured events at around 30 venues around the country.

Keith Edwards discusses the NIAB Transformation resource

One of these events was entitled 'Botany Live' and was led by Anne Osterrieder who is a lecturer in Biology and Science Communication at Oxford Brookes University as well as being the editor of the Annals of Botany blog (https://aobblog.com/). The aim of this event was to use the accessible online streaming resource Periscope to bring plant science into peoples' homes and workplaces. The organisers requested that people live-stream a 'short peek' into their lives as plant scientists.

Over the weekend of FoPD (May 18th-21st) 32 events signed up to provide a Botany Live video. The majority of these were from the US and UK but also featured videos from Lebanon and Argentina! These videos are being uploaded to the Botany Live website (https://botany.live/events/) so you can go back and check out the action!

Botany Live kicked off on the evening on May 17th (UK time) with Marcela Karey Tello-Ruiz who is the coordinator of the Gramene project in New York. She live- streamed a 'Plant Superpowers' session in which she interacted with a group of primary school students to share the joy of amazing plants! https://www.pscp.tv/w/1yoJMBLXagDxQ

Official FoPD day, May 18th, also saw the majority of Botany Live events. These were kicked off by Alison Bentley providing an introduction to the NIAB Innovation Farm facility prior to the Wheat Transformation Facility Wrap up meeting (see also page 38 in this edition of GARNish). This video also featured Keith Edwards, Ben Sibbett and Sinead Drea discussing their wheat research projects (https://www.pscp.tv/w/1nAKEBqpYolGL).

Our event dovetailed nicely with a video from Craig Sturrock and colleagues at

Introducing the University of Nottingham Hounsfield Facility

the University of Nottingham, who gave a tour of the Hounsfield CT scanning facility (https://www.pscp.tv/w/1BdGYvXqpYyJX). Throughout the (UK) afternoon there were a number of short videos from Kew Gardens that introduced some of the interesting plants that they have on site! A real video highlight was a very well choreographed livestream organized by Dr Jonathan Mitchley (aka Dr M) from the University of Reading in which he interacted with a group of school children, who gave their 'plant highlights' (https://www.pscp.tv/w/1BRKjWjmrYZGw)

Dr M goes Wild for Botany Live at the University of Reading

The livestream action then crossed the pond, featuring a Q+A from the Brilliant Botany blogger Claire (https://www.pscp.tv/w/1YpKknoyqAZxj), a tour of the ABRC laboratory facilities at Ohio State University (https://www.pscp.tv/w/1zqKVAzmDzVxB), as well as the greenhouse facilities at the Boyce Thompson Institute in Cornell. Later in the day, the livestream switched to focus on Argentinian Women Plant Scientists!

On Saturday May 20th researchers from the University of Glasgow featured in a really exciting set of videos from their FoPD celebration at Glasgow Botanic Gardens: http://www.periscopeforweb.net/m_papanatsiou

Truly it was a mixed bag of events and was an excellent first attempt at using this type of media to promote plant science. Hopefully Botany Live will be repeated again with more people taking up the challenge of putting together an interesting video for the global community……and tackling the connectivity issues relies on WiFi or a good 3G signal!

Botany Live was kindly supported by the SEB, the Annals of Botany Company, Plantae, the Quiet Branches and Oxford Brookes University.

Ensifer-mediated transformation: a novel technology platform for the generation of transgenic plants

Dheeraj Singh Rathore
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Ewen Mullins

Teagasc Crop Research Centre,

A team of researchers at Teagasc, Crops Research Centre and collaborators from the University College Dublin have discovered a novel technology platform to genetically engineer both monocot and dicot plant species. A non-Agrobacterium species entitled *Ensifer adhaerens* strain OV14 underpins the successful crop transformation protocol, termed as Ensifer-Mediated Transformation (EMT) technology. Initially proven successful on the model plant Arabidopsis, the host range of EMT continues to expand and now includes potato, rice, tobacco, cassava, safflower and oilseed rape. With collaborators we continue to focus on identifying new species receptive to EMT, with current efforts focussed on establishing robust protocols for cereals.

In 2012, EMT was first proposed as an efficient alternative to existing gene transfer systems by Wendt *et al*. (2012). This work reported the use of *Ensifer adhaerens* strain OV14; a soil-borne alpha-proteobacteria of the Rhizobiacae family. When equipped with the pCAMBIA5105 unitary plant transformation vector (Jefferson *et al*. 2006) *Ensifer adhaerens* strain OV14 demonstrated the ability to effectively transform the plant species' *Solanum tuberosum, Nicotiana tabacum* and *Arabidopsis thaliana*, with transformation efficiencies of 35.1, 20.9 and 0.12 %, respectively (Wendt *et al*. 2012). The study also demonstrated the capability of *E. adhaerens* OV14 to genetically enhance an existing potato variety with a commercially important trait, potato late blight resistance (Wendt *et al*., 2012).

Of recent significance for EMT technology has been the characterisation by Rudder *et al*. (2014) of the 7.7 Mb genome of *E. adhaerens* OV14 which we now know comprises of two circular chromosomes (3.96 Mb and 2.01 Mb) and two plasmids (1.61 Mb and 125 Kb). By comparing the genome sequence of *E. adhaerens* OV14 against the classic genetic engineer; *Agroabcterium tumefaciens* C58 and *Sinorhizobium meliloti* 1021 (a rhizobia with a propensity for low rates of genetic engineering; Broothaerts *et al*., 2005), it is clear that both *E. adhaerens* OV14 and *S. meliloti* 1021 possess homologs to all chromosomal-based genes cited as essential for *A. tumefaciens* induced genetic transformation. More interestingly, the genes that exert a positive influence on the ability to transform a plant genome were present in the genome of *E. adhaerens* OV14 but absent from *S. meliloti* 1021. The study further investigated the presence of vir genes (machinery essential for transformation) to reveal that there were analogues of three Ti plasmid based genes viz., virB4, virB11 and virD4 present in *E. adhaerens* OV14. Overall, the sequence analysis of the *E. adhaerens* OV14 genome has expanded our understanding of the molecular mechanisms that regulates successful transformation of plant genomes via EMT.

In addition to the genomic features discovered in *E. adhaerens* OV14, collated results from current EMT users continues to confirm the efficacy of EMT. For example, partners at the International Centre for Tropical Agriculture (CIAT) have successfully transformed three rice cultivars viz., Nipponbare, Curinga and the recalcitrant IR64 with transformation efficiencies of 16, 7 and ~1 %, respectively. Further, the T-DNA integration patterns within the rice genome via EMT were random throughout the rice genome and similar to that of AMT with partial or complete deletion of right or left borders or adjacent T-DNA (Zuniga-Soto *et al*. 2015).

Figure 1: Visualisation of the growth difference between the segregating T2 individuals (left 5 seedlings are kanamycin resistant KR and right 5 seedlings are kanamycin sensitive KS) germinated *in-vitro* in presence of 100 mg L-1 kanamycin.

E. adhaerens OV14 was originally isolated from the rhizosphere of oilseed rape and we have shown that it can also deliver transgenes into its original host *Brassica napus*, with a transformation efficiency of 4.0±0.021%, which was based on stable transgene integrations of 1–3 copies/line. Segregation analysis of the inserted nptII gene in the T2 generation indicated both Mendelian and non-Mendelian inheritance patterns for the designated kanamycin resistance phenotype (Fig. 1), thus confirming the stable transfer of the introgressed cassette (Rathore *et al*. 2016).

Separately, we continue to optimise the growth conditions for EMT, which includes the modification of growth media to address the pleomorphic trait of E. adhaerens OV14 (Rathore *et al*. 2015). Cultivation conditions have been optimised in order to achieve efficient rates of electroporation with plant transformation plasmids of up to 53 Kb in size. While *E. adhaerens* OV14 is resistant to ampicillin, paramomycin, streptomycin, spectinomycin, ticarcillin-clavulanate and kanamycin, phenotype screens confirm the strain's susceptibility to gentamicin (≥10 mg L-1), tetracycline (≥10 mg L-1), chloramphenicol (≥100 mg L-1) and cefotaxime (≥500 mg L-1). As part of our efforts to assist present and future users of EMT, we are expanding the toolkit to include alternative vectors and growth protocols, while investigating the potential of EMT to achieve non-genotype dependent gene transfer in target crop species.

Currently, our primary focus is on characterising how a plant cell responds to EMT as well as identifying the genetic processes that underpin the ability of *E. adhaerens* OV14 to achieve EMT. In parallel, we have generated a flexible licensing strategy that seeks to maximise the number of EMT users while ensuring users gain access to our latest developments in EMT. From a regulatory perspective has been recently clarified by the USDA APHIS who confirmed the non-plant pest status of Ensifer adhaerens OV14. We currently have 18 partners around the world evaluating EMT for their own specific purposes. If you wish to join the list please get in contact and we will explain our policies around ease of use. If you wish to initiate a research evaluation we have a range of flexible options available to suit both private and public institutions. After five years of the discovery of Ensifer-mediated transformation technology, the tale of Ensifer adhaerens has only begun. We encourage you to visit our web page www.emt4crops.com and join us in evaluating EMT on your crop of choice.

References

Jefferson RA, *et al* (2006) Freedom to co-operate: Transbacter as a Biological Open Source (BIOS) Tool for Gene Transfer. 8th international congress of plant molecular biology abstracts. Plant Mol Biol Rep 24(2):141-160

Rathore DS, *et al* (2016) Capability of the plant-associated bacterium, Ensifer adhaerens strain OV14, to genetically transform its original host Brassica napus. Plant Cell Tiss Organ Cult:1-10

Rathore DS *et al* (2015) Profiling antibiotic resistance and electrotransformation potential of Ensifer adhaerens OV14; a non-Agrobacterium species capable of efficient rates of plant transformation. FEMS Microbiol Lett 362(17)

Rudder S *et al* (2014) Genome sequence of Ensifer adhaerens OV14 provides insights into its ability as a novel vector for the genetic transformation of plant genomes. BMC genomics 15:268-285

Wendt T *et al* (2012) Production of Phytophthora infestans-resistant potato (Solanum tuberosum) utilising Ensifer adhaerens OV14. Transgenic Res 21(3):567-578

Zuniga-Soto E *et al* (2015) Ensifer-mediated transformation: an efficient non-Agrobacterium protocol for the genetic modification of rice. SpringerPlus 4(1):1-10

Spotlight on:

The National Institute for Agricultural Biology

Kindly compiled by Alison Bentley

NIAB (National Institute of Agricultural Botany) was established in 1919 to provide applied plant science innovations directly to the UK agricultural industry. Our traditional activities have focussed on science-led plant variety and seed characterisation, evaluation, quality control and knowledge transfer. In 2006 NIAB invested in a new translational research capability through the establishment of a Genetics & Breeding program. This program aims to deliver cutting-edge plant science innovations to plant breeding and production in the UK and globally. Working primarily on cereals, with expanding programs in legumes and vegetatively propagated crops, the Genetics & Breeding program encompasses pre-breeding, targeted dissection of quantitative traits, application of quantitative genetic information to breeding and a highly efficient crop transformation capacity for genetic modification of arable crops. With a mission to put plant science into practice our integrated program aims to help the agricultural sector fulfil its production potential.

Dr Alison Bentley
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As Director of Genetics and Breeding I have oversight of a group of 40-50 staff and students working on a range of exciting applied crop science projects. My main research interests are in the complex genotype x environment interactions controlling agriculturally significant traits. It is always a challenge to contextualise novel plant science discoveries (particularly in model species) and their potential to deliver impact to large-scale agriculture. Crop varieties take years to produce and test and are the product of intricate step-wise selection over multiple generations. New genetic discoveries, whether they be single cloned genes or novel epigenetic regulators must be incorporated into breeding programs in order to deliver impact in the form of improved seeds. These seeds must then withstand and thrive in a field environment, with variable resources and a plethora of biotic stresses in order to deliver income and secure food supply. In my projects at NIAB (including the recently funded Designing Future Wheat Cross-Institute Strategic Programme) the primary emphasis is on how to best exploit genetic diversity and understand positive and negatives trade-offs. I also work on a number of projects looking at nitrogen inputs into cereal production and how translational approaches can be used to both understand genetic mechanism and integrate this with performance within farming systems in the UK and the developing world.

Alison Bentley

Bentley: Field-scale testing of the nitrogen response of diverse

Professor Ian Mackay
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Ian Mackay

My research focuses on the application of methods from statistical, population and quantitative genetics to improving the efficiency of plant breeding. At NIAB we have developed multi-parent advanced generation intercross (MAGIC) populations for fine mapping in winter wheat and championed the use of these populations in other crops. These large diverse populations have greater precision and more power than found in bi-parental populations. For trait mapping, we are also involved in association mapping projects in wheat and barley. We strongly advocate adoption of replication studies in independent mapping populations to validate QTL discovery. MAGIC populations and association mapping panels vcomplement each other well for this purpose.

We were early proponents of genomic selection in crops and collaborate with public and private sector breeders, nationally and internationally in this area. An important component of our activity is teaching. We have run a two week intensive course, “Quantitative Methods in Plant Breeding”, annually since 2008. This same course has been taught in France, India, Malaysia and Australia: there is an increasing recognition of the importance of quantitative methods in integrating genomics information into breeding programmes in a cost effective and efficient manner.

Dr Emma Wallington
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Emma Wallington

I lead the Crop Transformation programme at NIAB. We provide crop transformation capability for many research projects, both within NIAB and the wider community – interacting both with academic researchers and commercial companies. We focus on transformation of crop plants such as wheat, barley, oilseed rape and rice and also the development of tissue culture and transformation systems. One of our current BBSRC funded projects, the Community Resource for Wheat Transformation (see page 34 in this edition of GARNish), has enabled us to interact with a large number of plant scientists who are more comfortable working with Arabidopsis or other model species. This 5 year funded project has enabled them to transfer their research into wheat, by providing 50 free wheat transformation slots, in addition to molecular and bioinformatic support from the team. The project has also allowed us to extend the range of winter and spring wheat varieties which can be transformed – providing a unique resource for researchers with disease candidate resistance genes to test, and making this research relevant to UK agriculture. We are currently preparing for a CRWT workshop and

Wallington: GM winter wheat produced at NIAB

looking forward to hearing the progress that our applicants have made with a diverse range of applications covering biotic and abiotic stress, or yield component traits in wheat. (see a report from the meeting elsewhere in this edition of GARNish). Other current projects focus on particular diseases and resistance mechanisms, nitrogen uptake and remobilisation or gene editing in wheat, the interaction of arbuscular mycorrhizal fungi with rice roots, and oil characteristics in oilseed rape. This is a very varied portfolio of projects, linked by the need for efficient and high throughput transformation systems, which we have put in place at NIAB.

Dr Phil Howell
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Our research focuses predominantly on pre-breeding with the major cereal crops wheat and barley. Pre-breeding involves the transfer of potentially useful genetic diversity from a range of unadapted donor sources into elite varieties through crossing, inbreeding and selection. Pre-breeding germplasm is typically evaluated in the field with the best lines transferred into commercial breeding programmes, and we have research collaborations with all the major cereal breeding companies. In our work, we have used mutants, overseas or obsolete varieties and landraces as potential donors, but have done a huge amount with the close diploid and tetraploid relatives of wheat, which we exploit through resynthesis and interploidy crossing. This is opening up exciting leads for traits such as yield, nitrogen-use efficiency, disease resistance, abiotic stress response, and end-user quality. We have also developed sets of +/- near-isogenic lines which can be used to study particular characters, eg starch mutations, grain pigmentation, presence/absence of awns, glaucous/non-glaucous canopy.


Phil Howell


Howell: Increasing wheat yield components (right) via pre-breeding with wild relatives

Dr Kay Trafford
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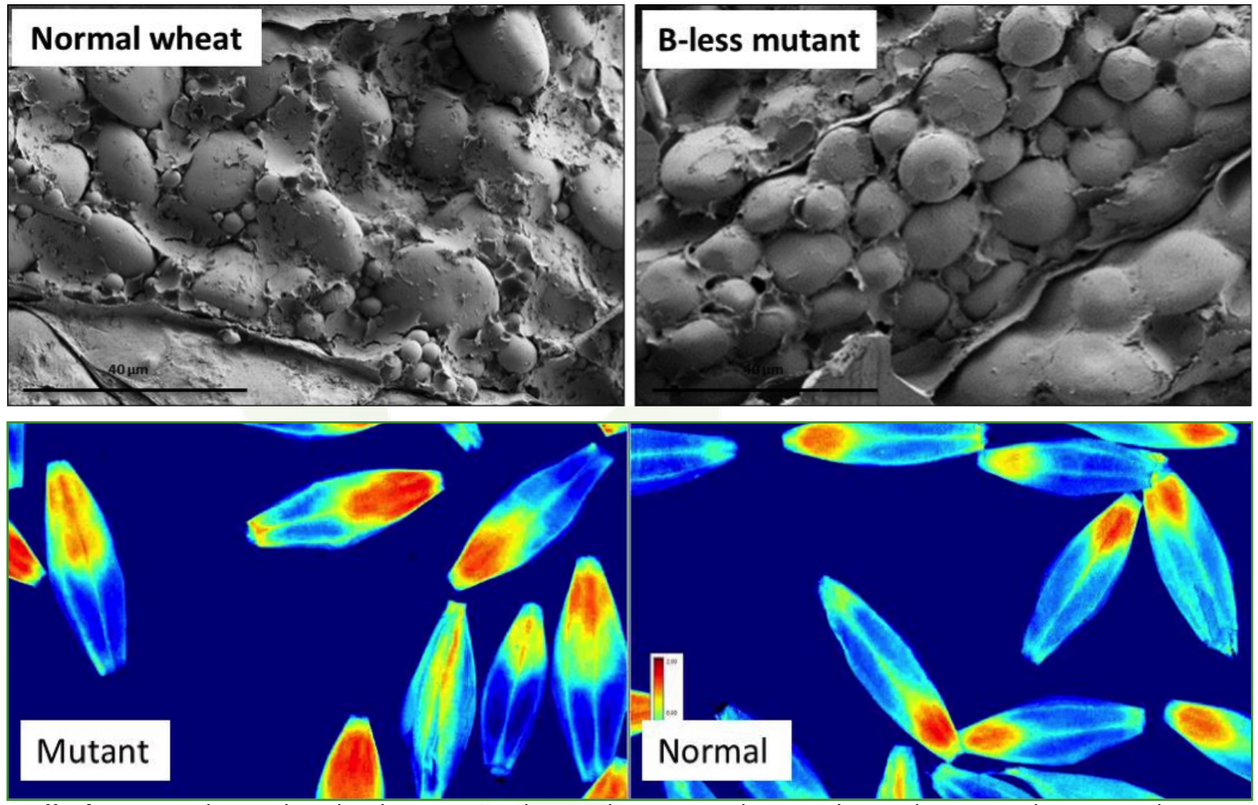
Our interests are in the biochemistry, molecular biology and evolution of starch biosynthesis in plants, particularly cereal grains. We have two primary projects in this area at present:

- B-granule project. Our aim is to understand the molecular and biochemical mechanisms that determine starch granule size and shape in wheat, barley and oats. Our ultimate goal is to identify and manipulate the gene responsible for the control of B-granule content, Bgc-1. As part of a BBSRC CIRC-funded project, we have located the Bgc-1 gene in Aegilops by fine-mapping and in parallel, produced B-granule-less lines of wheat for functionality testing by selecting and stacking deletions of the Bgc-1 homoeologous regions


Kay Trafford



Trafford: Variation for starch and embryo size in wheat. In lower image larger embryos (shown in red) in mutant lines were imaged using a multispectral imaging videometerlab analyser (Adrian Waltho analytik.co.uk)

- Large embryo project. The aim of this project is to identify the genetic basis of the large embryo phenotype in four independent mutants of barley with lesions at the Lys3 locus. Our goal is to identify the Lys3 gene by positional cloning in order to study it at a molecular level.

This will enhance our understanding of the control of embryo size in cereal grains. Ultimately, this knowledge may enable separation of the favourable (nutritional enhancements) from the unfavourable (yield depression) traits of the lys3 phenotype.

Dr Eric Ober
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Our research focuses on improving crop yields through increased understanding of plant traits that contribute to yield formation, drought tolerance and water use efficiency. Research is funded by government and the agricultural industry, and work is done in close collaboration with commercial plant breeders. Emphasis is placed on translating fundamental understanding of plant biology into practical field-scale screening methods,


Eric Ober

and identifying superior germplasm using advanced phenotyping methods, including drone-based remote sensing. Current projects include: genomic selection in wheat; selection methods for improving photosynthetic efficiency; developing a growth guide and crop model to optimise oat growth and grain quality; developing a model-based N management tool for baby corn production in India; an EU-funded project that compares landrace and elite varieties of wheat and barley for low input and reduced tillage systems. A new project in collaboration with Cambridge University's Institute for Manufacturing seeks to develop a tool for laser ablation tomography for examining plant tissue microstructure. Expertise is in the areas of crop physiology, field phenotyping, plant water relations, irrigation efficiency, regulation of growth and grain development during water deficit, and hormonal regulation of root growth and osmotic adjustment.

Dr Sarah Dyer
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Our research interests are centred on the exploration of genetic diversity for crop species and their wild relatives, and the application of this information to assist breeding for agronomically important traits. Genomics can help to focus breeding efforts, with targets linked to sustainable agriculture in the context of limited resources and challenges emerging from climate change. Improving tools to enable interaction with and exploration of large datasets is essential to enable the identification of materials of interest which could be incorporated into breeding programs.

Sarah Dyer

We have a particular interest in cassava (*Manihot esculenta*), a globally important staple for >500 million people in the tropics. Working with the cassava germplasm collection in the International Center for Tropical Agriculture (CIAT), Colombia, we are applying genomics and bioinformatics approaches to explore the structure of the collection and identify variations linked to pests and disease, pressures which are predicted to increase with changing climates. We are keen to ensure that data which we generate are made available appropriately and are developed into useful resources for the community.

Dr Tom Wood
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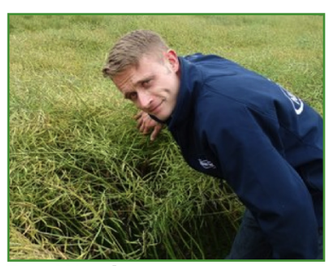
Our research is focussed on improving understanding of crop pathogens and host resistance in order to mitigate negative effects on yield and quality. We work largely with pathogens affecting oilseed rape (*Brassica napus*) and Faba bean (*Vicia faba*), we utilise classical pathology, genetics, genomics and transcriptomics to investigate pathogen population dynamics, study host-pathogen interactions, for mapping genes and to develop new tools for diagnosis and detection.

Our aim is to enhance diagnostic capabilities for identifying new pathotypes and changes in virulence in order to aid disease management strategies. We are currently developing loop-mediated isothermal amplification (LAMP) methods to provide fast, in-field detection for a range of pathogens affecting UK arable and horticultural crops. Recent diversity studies in the lab



Tom Wood

Wood: Sequencing fungal genomes on the Oxford Nanopore Technologies MinION

have identified a dichotomy within UK/European populations of the recently-emerged oilseed rape pathogen *Verticillium longisporum*; this contradicts initial hypotheses that the disease was introduced on commercial oilseed rape produced in Continental Europe and has clear implications for future management practices.

Dr Sarah Holdgate
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Our main interest is in plant diseases that affect cereal production in the UK. One specific aspect of our work is the threat from changes in the populations of previously established plant pathogens. The best example of this is surveillance work carried out as part of the UK Cereal Pathogen Virulence Survey. This project has just celebrated its 50th anniversary and monitors the populations of the pathogens causing wheat yellow rust, wheat

Sarah Holgate

brown rust, and wheat and barley powdery mildew. Many changes have occurred over the past 50 years, however none quite as big as an exotic incursion of *Puccinia striiformis f.sp. tritici (Pst)*, causal agent of wheat yellow rust in 2011. Initially named as the "Warrior" race, this sudden arrival quickly dominated the UK population.

Another interesting change in population comes from the causal agent of Septoria leaf blotch, *Zymoseptoria tritici*. Isolates in the UK vary greatly in their sensitivity to key fungicide active ingredients, however a recent change in virulence to the wheat variety Cougar has prompted us to investigate varietal adaptation of isolates in the subsequent seasons. The origins of these new variants, along with the risk posed to existing UK wheat varieties are all currently under investigation.

NIAB Wheat Transformation Resource Wrap-up Event

Geraint Parry

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In 2012 the BBSRC funded a Bioinformatics and Biological Resources Fund project entitled 'A COMMUNITY RESOURCE IN WHEAT TRANSFORMATION (CRWT)' that was led by Dr Emma Wallington at the National Institute for Agricultural Botany (NIAB). This resource proposed to make their in-house expertise in wheat transformation available to the UK plant science community. Over the length of the grant they have hosted five calls in which UK-based researchers could propose a gene of their interest to be introduced into hexaploid wheat. Successful applicants provided the clone to the CRWT facility before the transformation was conducted at NIAB. The T1 wheat plantlets were then returned to the host labs for further analysis, with continued support from the experts at NIAB.

The available transformation spaces were designed to be split between researchers who work on crops and those that usually work in other model organisms but who want to transfer their research into wheat. The full list of funded projects is can be found here: http://www.niab.com/pages/id/399/Accepted_Applications_in_the_CRWT_Project. 41 (of 50) projects have now been completed, some have been very successful whereas others haven't worked out as well… basically the story of science writ large!

As the grant is coming to an end, the NIAB Innovation Farm (https://www.innovationfarm.co.uk/) hosted a wrap-up event to showcase some of the successful projects. This involved a day of plenary lectures alongside a poster session that included plenty of opportunity for discussion peoples experience interacting with the CRWT facility. As highlighted in the Wheat Training article earlier in this issue of GARNish (see page 8), simply growing wheat is not so trivial when you are only familiar with working with Arabidopsis!

Importantly two of the talks discussed work from the CRWT that has led into follow-on research that is now funded by current BBSRC Sustainable Agriculture Research and Innovation Club (SARIC) research grants. Every researcher knows the often-frustrating challenge of acquiring pre-data in order to obtain a grant on a follow-on topic. In that situation it is extremely difficult for a research group who works exclusively with Arabidopsis to obtain the data to move their work into wheat or other crop species, even if their ideas are scientifically sound. In these situations the resource provided by CRWT can be invaluable in generating data that adds to future grant applications.

Lee Hunt works with Julie Gray at the University of Sheffield and described work from a SARIC grant entitled '*Reduced Stomatal Density Wheat: New Prospects for Drought and Pathogen Resistance*', which follows on from a large portfolio of Arabidopsis research on similar topics that has been conducted in the Gray lab. In collaboration with the CRWT they were able to generate wheat plants that have fewer stomata and are currently investigating whether this affects the ability of Septoria species pathogens to infect these plants during different climatic conditions.

Cara Griffiths from Rothamstead Research described a current research project that is looking at effect of introducing a gene from the resurrection plant *Sporobolus stapfianus* into wheat. Cara's previous excellent work had showed that introducing this gene (SDG8i, encoding a Group 1 glycosyltransferase (UGT) into Arabidopsis causes striking drought resistance without exhibiting a yield penalty (Islam, Griffiths

Cara Griffiths discusses her work that transfers a gene from resurrection grass into both Arabidopsis and wheat.

et al (2013) PLoS ONE https://doi.org/10.1371/journal.pone.0080035). This work leads into another SARIC grant obtained by Matthew Paul at Rothamsted that is focussed on identifying factors that improve drought tolerance in wheat. The usefulness and expertise of the CRWT resource is highlighted by the fact researchers from Rothamsted choose to use this resource situated at NIAB.

It was gratifying to learn that the researchers who interacted with CRWT were from 19 institutions from all around the UK. Ben Sibbett is a PhD student at the University of Southampton and presented two projects that are supervised by Matthew Terry and aim to investigate the interaction between light and GA signalling by generating wheat plants that have reduced function of PIF3 and PIF4. These projects are in their early stages but the current data already suggests that altering levels of PIF proteins can alter plant height by changing the activity of the RHT1 DELLA protein. If shorter crop varieties can be generated these results may have future implications for global food security...if the plants still retain their current yield levels.

At the start of the day Rhian Howells and Melanie Craze from NIAB provided details of what they have learnt during the course of the CRWT grant. Melanie provided an overview of the facility that has now produced over 1500 transgenic wheat plants, approximately 50% of which include a gene taken from non-crop model organisms. Interestingly when using Fielder Wheat they found a wide variety in transformation frequency (between 24%-63%). This variation is thought to be dependent on the function of the inserted genes rather than due to any component of the transformed construct.

Rhian has been using the CRISPR-Cas9 system in an attempt to generate gene-edited wheat. They have been extremely successful in obtaining T2 edited wheat lines that both have no off-target effects and have had the Cas9 enzyme crossed out of the population. Rhian stated that they can go from initial transformation to a transgene-free edited wheat in 36 weeks. This relatively rapid turnaround time could be a real game-changer in the communities ability to produce edited plants that might be free from existing GM regulations. However as a note of caution both Professor Claire Halpin (James Hutton Institute) and Professor Keith Edwards (University of Bristol) provided different and less-straightforward stories regarding their labs abilities to generate transgene-free edited plants. This highlights that our understanding of the factors that allow effective and consistent gene-editing is still in its infancy. GARNet looks forward to hosting a conference in this research area in 2018. Watch this space for more details!

Overall the CRWT has clearly provided an outstanding resource for those researchers who do not have easy access to wheat transformation facilities. This is particularly important as the funding landscape moves from basic to applied research. The funding for the CRWT ends later in 2017 and it is hoped that follow-on support is soon forthcoming as there is clearly a need for this facility from within the plant science community. This might be particularly relevant given NIABs seeming mastery of CRISPR-Cas9 mediated gene editing in wheat!

SOCIETY FOR EXPERIMENTAL BIOLOGY PRESENTS:

NEW BREEDING TECHNOLOGIES IN THE PLANT SCIENCES – APPLICATIONS & IMPLICATIONS IN GENOME EDITING

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7 – 8 JULY 2017
UNIVERSITY OF GOTHENBURG, SWEDEN

ORGANISED BY
RUTH BASTOW, GLOBAL PLANT COUNCIL,
GERAINT PARRY, GARNet,
STEFAN JANSSON, UMEÅ UNIVERSITY, SWEDEN AND
BARRY POGSON, AUSTRALIAN NATIONAL UNIVERSITY, AUSTRALIA

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- INTERACTIVE WORKSHOP SESSIONS AIMED AT OPTIMISING YOUR EXPERIMENTS USING CRISPR-CAS9
- DEBATE THE GLOBAL IMPLICATIONS OF NEW BREEDING TECHNOLOGIES WITH POLICY AND LEGISLATIVE EXPERTS
- HEAR ABOUT EXCITING CRISPR-CAS9 SUCCESS STORIES FOR GENE-EDITING IN PLANTS
- ENGAGE WITH SCIENCE COMMUNICATORS TO LEARN HOW TO TACKLE THE DIFFICULT ISSUES SURROUNDING THE USE OF GENE-EDITING TECHNOLOGIES.

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