GARNet Gene Editing Workshop

University of Bristol
March 26th-27th 2018
Welcome

The GARNet Advisory Committee are delighted to welcome you to this workshop on plant gene editing. I hope you agree that we have brought together a world-leading selection of speakers who will introduce the technical challenges and policy issues that surround the use of gene editing technology.

We have kept this meeting to a small number of delegates to encourage discussions and debate, particular during the open discussions and at the poster sessions. Hopefully new collaborations can be born over these two days!

GARNet is a BBSRC-sponsored network that aims to ensure the UK plant research community remains competitive and productive at the national and international level by helping researchers make the best use of available funding, tools and resources. Therefore by hosting this workshop we are supporting the use of this new exciting technology.

This workshop would not be possible without the support of the Bristol Centre of Agricultural Innovation and the New Phytologist Trust.

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The GARNet Coordinator Geraint Parry is based at Cardiff University. For more information please see the GARNet website at www.garnetcommunity.org.uk, and the GARNet blog at http://blog.garnetcommunity.org.uk

You can also follow us on Twitter on @GARNetweets

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Over the coming months please look out for articles appearing in New Phytologist from workshop participants.

High Value Chemicals from Plants
Meeting Information

Please see facing pages for Meeting locations. More information about travel to Bristol can be found at: https://garnet-ge-workshop.weebly.com/accomodation-and-transport.html

Connectivity:

The University of Bristol is serviced by the EduRoam network but if you need a guest WiFi ID then please ask at registration.

Catering, Poster Session and Evening Reception

Registration, tea breaks and lunch will be held in the School of Chemistry, University of Bristol, Cantock’s Cl, Bristol BS8 1TS.

If you have informed us of any dietary requirement then those meals will be labelled but if you have any queries then ask at registration.

Meeting Venues:

The presentations and poster session will take place at the Department of Chemistry, Cantock’s Cl, Bristol BS8 1TS. We encourage attendees to put up their posters during the registration session prior to the first session.

Conference Dinner:

The Conference dinner takes place in Riverstation, The Grove, Bristol BS1 4RB. Dinner will be served at 8pm but we encourage people to arrange early to have a introductory drink.

Taxis

If you require an incoming taxi then one can be picked up at the station. Otherwise please ask the organisers to book a taxi for you.

Social Media:

We encourage you to share the meetings exciting science with the wider community so the twitter hashtag is #GARNetGE18
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### Monday March 26th

**9am: Meeting Registration and Poster setup**  
*School of Chemistry*

**9.30am:** **Opening Plenary:** Professor Stefan Jansson (UMEA Plant Science Centre): *Cooking (and eating) the first gene-edited meal!*

**10.15am:** Tea Break

**Session I: Gene Editing in Dicots**  
*Chair: Dr Jill Harrison*

**10.45am:** Vladimir Nekrasov (Rothamsted Research): *Genome editing: a revolutionary tool for basic and applied plant science*

**11.30am:** Michaela McGinn (Illinois State University): *Gene Editing in Pennycress*

**12.00pm:** Lunch with posters

**1.00pm:** Andreas Weber (HHU Dusseldorf): *Cas9-based genome editing in Arabidopsis thaliana: Toward synthetic evolution of C₄ photosynthesis*

**1.30pm:** Baptiste Castel (John Innes Centre): *Optimizing CRISPR-Cas9 mutagenesis to study immunity in Arabidopsis thaliana*

**2.00pm:** Julia Richter (BOKU, Vienna): *Multiplex mutagenesis of four clustered CrRLK1L with CRISPR/Cas9 exposes their growth regulatory roles in response to metal ions*

**2.30pm:** Extended Q+A with Invited Speakers

**3.00pm:** Tea Break

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### Monday March 26th

**Session II: Gene Editing in Monocots**  
*Chair: Dr Heather Whitney*

**3.30pm:** Keith Edwards (University of Bristol): *Adventures with Wheat Gene Editing*

**4.00pm:** Yiping Qi (University of Maryland): *Use of CRISPR-Cpf1 for plant gene editing*

**4.30pm:** Emma Wallington (NIAB): *Gene editing in Cereal Crop species*

**5.00pm:** Extended Q+A with Invited Speakers

**5.30pm:** **Keynote Plenary:** Ben Davies: Transgenic Core Head, Wellcome Trust Centre for Human Genetics, University of Oxford: *CRISPR/Cas9 site-specific nuclease for functional analysis in the mouse*

From 7.30pm: Reception Drinks at Riverstation, The Grove, Bristol BS1 4R

**8pm:** Conference Dinner at Riverstation
**Tuesday March 27th**

8.30am: Morning Coffee  
Chair: Professor Keith Edwards

**Session III: Gene Editing and Global Regulatory Landscape**

9.00am: Dennis Erickson (SLU): *Reviewing the current EU regulatory environment for use of gene editing technology*

9.45am: Gary Marchant (Arizona State University): *Risk based approaches for the regulation GE crops*

10.30pm: Tea break

11.00am: Louise Ball (DEFRA): *Should genome-edited organisms be classed as GMOs: a UK perspective?*


12.00am: Extended Discussion session

12.30pm: Lunch with posters

**Tuesday March 27th**

**Session IV: Novel uses of gene editing technologies**

Chair: Dr Geraint Parry

1.30pm: Fabien Nogue (INRA Centre de Versailles): *Gene editing in Physcomitrella patens*

2.00pm: Heather Whitney (University of Bristol): *Nanomaterials for the delivery of gene-editing in plants*

2.30pm: Thomas Birkett (University of Warwick): *Expression of CRISPR using Viral Vectors for Targeted Mutagenesis.*

2.45pm: Choun-Sea Lin (Academia Sinica, Taipei): *Application of protoplast technology to CRISPR/Cas9: single protoplast isolation and protoplast regeneration in tobacco*

3.00pm: Alex Leydon (University of Washington, USA): *The GA-biosynthesis pathway can be re-programmed in a model driven manner using hormone activated Cas9-based repressors (HACRs)*

3.30pm: Extended Q+A with Invited Speakers

4.00pm: Meeting End
Cooking (and eating) the first gene-edited meal!

Stefan Jansson

Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, 901 87 Umeå, Sweden

In November 2015, The Swedish Board of Agriculture announced their opinion that Arabidopsis plants that have been modified using CRISPR-Cas9 where DNA where only a piece of a genes has deleted and but no novel DNA added, not fall within the scope of the GMO legislation. This historical decision opened up for the use of genome-edited deletion mutants in agriculture. In the summer of 2016, such genome-edited Brassica plants was grown in a garden in Bjurfors, Sweden, under rather primitive conditions and on August 16, for (probably) the first time ever, field-grown CRISPR-mutants were harvested and cooked.

The meal got significant attention and has so far been reported in ca 300 media in ca 40 countries. In this talk, the reasoning behind and the various kinds of responses and follow-ups to the meal will be described. Special focus will be put on the consequences of the decision and the meal, in particular intriguing issues around the fact that there will now be plants grown in Sweden (and other parts of the world) which are not covered by the GMO legislation, while the legal status of the very same plants in other EU countries is still unclear. This legal limbo challenges core values of EU like free movement of goods, and pinpoints the inability of EU to adjust its legislation around GMOs to the development in science and technology.
Genome editing: a revolutionary tool for basic and applied plant science

Vladimir Nekrasov

Rothamsted Research, United Kingdom

Genome editing is a rapidly expanding field in plant and animal systems with CRISPR/Cas being the tool of choice for introducing changes into genomic DNA in a precise manner. CRISPR/Cas, in its conventional form, is based on Cas9, an RNA-guided DNA endonuclease, that can be targeted to a genomic sequence of choice by engineering a separately encoded single guide RNA (sgRNA), with which it forms a complex. CRISPR/Cas target specificity is encoded in the 20 bp guide sequence of sgRNA that can be easily engineered to recognise a particular DNA sequence.

By now, CRISPR/Cas has been successfully applied in many model and crop plants as a tool for gene function studies as well as for improvement of various agriculturally important traits. CRISPR/Cas has proven to be highly specific in plants creating minimum numbers of off-target mutations in plant genomes. At the moment, there is an ongoing debate in the EU and other parts of the world on whether genetically edited crops, which carry mutations indistinguishable from naturally occurring ones, should be exempt from the GMO regulation.
Gene Editing in Pennycress

Michaela G. McGinn¹, Evan Johnson², Brice Jarvis¹, Malihe Esfahanian¹, Tara Nazarenus³, M. David Marks², Edgar B. Cahoon³, and John C. Sedbrook¹

1- School of Biological Sciences, Illinois State University, Normal, IL 61790
2- Department of Plant biology, University of Minnesota, St. Paul, MN 55108
3- Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68588

Pennycress (Thlapsi arvense) is a member of the Brassicaceae family closely related to Arabidopsis and rapeseed canola that holds considerable agronomic and economic potential as a winter annual oilseed cover crop. Pennycress possesses a unique combination of attributes including extreme cold tolerance, rapid growth, over-wintering growth habit, and a natural ability to produce copious amounts of seeds high in oil and protein. Pennycress could generate billions of liters/tons of oil and seed meal annually throughout temperate regions of the world for food, feed, and industrial applications without displacing established crops or requiring land use changes. For example, pennycress can be grown as a winter cover crop between corn and soybeans on otherwise vacant farmland, providing ecosystem service benefits including reduced soil erosion and reduced nutrients runoff.

The diploid genome of pennycress has been sequenced, revealing it shares 86% sequence identity with the Arabidopsis genome allowing straightforward translation of knowledge gathered from decades of Arabidopsis research. We have developed an Agrobacterium-mediated floral dip transformation method and have successfully used the CRISPR-Cas9 genome editing technique to generate loss-of-function mutations in genes of agronomic importance including those affecting seed oil fatty acid composition and seed coat fiber content.

Here we will display our work with CRISPR-Cas9 in pennycress and discuss related insights including common pitfalls of the CRISPR-Cas9 system.
Cas9-based genome editing in \textit{Arabidopsis thaliana}: Toward synthetic evolution of $C_4$ photosynthesis

Hahn F, Mantegazza O, Eisenhut M, Schlüter U, Andreas Weber

Institute of Plant Biochemistry, Cluster of Excellence on Plant Science (CEPLAS), Heinrich Heine University, Düsseldorf, Germany

$C_4$ photosynthesis has independently and convergently evolved approx. 70 times in flowering plants. Most $C_4$ plant species display a distinct leaf anatomy (Kranz-anatomy) and similar metabolic and biochemical features. Computational modeling indicates that $C_4$ photosynthesis evolves from $C_3$ photosynthesis via $C_3$-$C_4$ intermediate states \cite{1,2}. $C_3$-$C_4$ intermediacy evolves via simple loss of function mutations and causes a nitrogen disbalance between leaf mesophyll and bundle sheath cells. Overcoming this nitrogen disbalance requires the shuttling of organic acids between mesophyll and bundle sheath cells, which under permissive conditions drives the system towards $C_4$ photosynthesis \cite{3}. Genetic hybrids between $C_3$ and $C_3$-$C_4$ intermediate species segregate for subtraits of $C_3$-$C_4$ intermediacy and thereby enable the identification of key enablers by quantitative genetics. Identification of such enablers is a prerequisite for engineering of the $C_4$ trait into $C_3$ species by synthetic biology approaches \cite{4}.

We have recently identified such enablers. These include cis-regulatory elements in the promoters of genes encoding components of photorespiration. To experimentally test these enablers in a $C_3$ genetic background and to eventually recapitulate the evolutionary trajectory from $C_3$ to $C_4$ photosynthesis in the model plant \textit{A. thaliana}, we have developed a non-invasive visual marker that enables detection of Cas9-activity in planta \cite{5,6}. We have used this maker system to test different concepts for homology-based gene editing and have developed a reliable system for homology-directed repair in \textit{Arabidopsis} \cite{7}. These protocol will now be employed for introduction of $C_4$-enablers into \textit{Arabidopsis}.

\begin{thebibliography}{9}
\bibitem{1} Heckmann et al. (2013) Cell 153: 1579-1588
\bibitem{3} Mallmann et al. (2014) eLife, 3: e02478
\bibitem{4} Schuler et al. (2016) Plant J 87: 51-65
\bibitem{7} Hahn et al. (2018) bioRxiv 243675; doi: https://doi.org/10.1101/243675.
\end{thebibliography}
Optimizing CRISPR-Cas9 mutagenesis to study immunity in *Arabidopsis thaliana*

Baptiste Castel¹, Laurence Tomlinson¹, Federica Locci², Jonathan DG Jones¹

1- The Sainsbury Laboratory, Norwich Research Park, Colney Lane, NR4 7UH, Norwich
2- Department of Biology and Biotechnology “Charles Darwin”, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

Using synthetic guide RNAs, (sgRNAs), the Cas9 nuclease can be directed to generate indels, DNA insertion and deletion in vivo at target sequences that carry a “protospacer-adjacent motif” (PAM). We conducted a systematic comparison of CRISPR-Cas9 constructs to optimize the method for Arabidopsis. We found that the Cas9 allele, the promoter that regulates Cas9 expression and the sgRNA greatly influence the overall efficiency.

In optimized constructs, we obtained high rates of stable homozygous mutants in the first generation after transformation. We have applied CRISPR to knockout immunity-related gene candidates whose mutants are not available from the public collections. We thus confirmed the role of a TIR-NLR-encoding gene in White Rust Resistance in Ws-2 and identified two redundant genes involved in Effector-Triggered Immunity.

Our results (i) highlight the convenience of CRISPR to study genetic traits when natural variation is not available and (ii) demonstrate that some combinations of CRISPR components enable high efficiency generation of stable mutants in one generation.
Multiplex mutagenesis of four clustered CrRLK1L with CRISPR/Cas9 exposes their growth regulatory roles in response to metal ions

Julia Richter¹, James Matthew Watson²,³, Peter Stasnik¹, Vera Schoft², Marie-Theres Hauser¹

1- Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences (BOKU), Muthgasse 18, 1190 Vienna, Austria
2- Vienna Biocenter Core Facilities GmbH (VBCF), Dr. Bohrgasse 3, 1030 Vienna, Austria
3- Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna BioCenter (VBC), Dr. Bohrgasse 3, 1030 Vienna, Austria

Resolving functions of closely linked genes is challenging or nearly impossible with classical genetic tools. Four members of the Catharanthus roseus receptor-like kinase 1-like (CrRLK1L) family are clustered on Arabidopsis chromosome five. To resolve the potentially redundant functions of this subclass of CrRLK1Ls named MEDOS1 to 4 (MDS1 to 4), we generated a single CRISPR/Cas9 transformation vector using a Golden Gate based cloning system to target all four genes simultaneously.

We introduce single mutations within and deletions between MDS genes as well as knock-outs of the whole 11 kb gene cluster. The large MDS cluster deletion was inherited in up to 25% of plants lacking the CRISPR/Cas9 construct in the T2 generation. In contrast to described phenotypes of already characterized CrRLK1L mutants, quadruple mds knock-outs were fully fertile, developed normal root hairs and trichomes and responded to pharmacological inhibition of cellulose biosynthesis similar to wildtype.

Recently, we demonstrated the role of four CrRLK1L in growth adaptation to metal ion stress. Here we show the redundant involvement of MDS genes in response to Ni²⁺ during hypocotyl elongation and to Cd²⁺ and Zn²⁺ during root growth. Our finding supports the model of an organ specific network of positively and negatively acting CrRLK1Ls.
CRISPR/Cas9 targeted mutagenesis is revolutionising the genetic manipulation of a range of crops including bread wheat. Recently groups in China and the UK reported the successful targeted mutagenesis of individual wheat genes thus confirming CRISPR/Cas9 as a viable technology for the manipulation of hexaploid wheat. Via the Bristol Synthetic Biology Centre (BrisSynBio; http://www.bristol.ac.uk/brissynbio/), we have used various CRISPR/Cas9 constructs to manipulate wheat, specifically with regards to genes involved in meiotic recombination.

Using both human and rice codon optimised Cas9 constructs our attempts to target the three TASpo11 homoeologues in wheat were unsuccessful. Analysis of the Cas9 transcripts generated from both the human and rice constructs indicated that, in hexaploid wheat, both were miss-spliced, and both failed to generate full length transcripts. Using the lessons learned from our early work, we designed a wheat codon optimised Cas9 construct that included a porcine 2A processing peptide and a wheat optimised GFP reporter gene. This construct, which should generate a single transcript but two separate proteins (Cas9 and GFP), both targeting the nucleus, has now been used to transformed, via particle bombardment, into wheat along with guide RNAs for four separate genes; TaGa20oxidase, TaMsh2, TaSpo11 and TaFancm.

In my presentation I will provide an up to date account of the edits observed and I will make some suggestions as to how one might carry out large scale targeted mutagenesis of hexaploid wheat.
Use of CRISPR-Cpf1 for Plant Genome Editing

Yiping Qi

Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland 20742, USA

Clustered regularly interspaced short palindromic repeats (CRISPR)-Cpf1 has emerged as an effective genome editing tool in animals. Here we compare the activity of Cpf1 from Acidaminococcus sp. BV3L6 (As) and Lachnospiraceae bacterium ND2006 (Lb) in plants, using a dual RNA Polymerase II promoter expression system. LbCpf1 generated biallelic mutations at nearly 100% efficiency at four independent sites in rice T0 transgenic plants.

Whole-genome sequencing of edited plants revealed no off-target mutations, suggesting Cpf1 is very specific for precise genome editing. Moreover, we repurposed AsCpf1 and LbCpf1 for efficient transcriptional repression in Arabidopsis, and demonstrated reduction of target gene transcription >10-fold. Our data suggest promising applications of CRISPR-Cpf1 for editing plant genomes and modulating the plant transcriptome.

Keywords: CRISPR, Cpf1, plant genome editing, transcriptional repression
Gene editing in Cereal Crop species

Emma J. Wallington

NIAB, Huntingdon Road, Cambridge CB3 0LE, United Kingdom
emma.wallington@niab.com

The ability to create precise modifications in a specific target gene using new breeding technologies such as gene editing has revolutionised studies of gene function in living cells. The NIAB Crop Transformation facility is able to provide transformation of a number of important crop species including cereals such as wheat, barley and rice and has been using CRISPR/Cas9 in a number of project collaborations.

Our recent results from rice and wheat projects will be described to demonstrate the effectiveness with which stable edits can be created and highlight some of the difficulties which can be encountered, particularly when working with complex genomes such as wheat. The hexaploid nature of wheat provides us with the opportunity to examine potential off-target effects, and the challenge to knock out all six copies in one genome.
Ben Davies

Wellcome Trust Centre for Human Genetics, University of Oxford

Site-specific nucleases, such as CRISPR/Cas9, have had an enormous impact on functional gene analysis. We are now able to manipulate the genome of experimental cells and organisms with unprecedented speed and accuracy, providing a powerful means of interrogating gene function.

We are using the technology in the mouse zygote to introduce human disease mutations into their orthologous positions within the mouse genome. The resulting mouse models of human disease provide an opportunity to investigate the biology underlying the disease and the models themselves may serve to test novel therapeutic and diagnostic strategies. I will present a couple of examples of this approach for protein coding mutations and will introduce our mouse production pipeline, focussing on technological improvements that we have introduced to maximize the efficiency of mouse model production.

As well as protein coding mutations, it is becoming clear that variation in non-coding DNA plays an important role in genetic disease. Investigating how non-coding DNA elements such as enhancers and promoters interact is essential to understanding how non-coding mutation may perturb gene expression networks, which might underlie disease pathology. We are using CRISPR/Cas9 site-specific nucleases in the mouse zygote to ablate binding sites for the CTCF/Cohesin complex within non-coding DNA. This is revealing how enhancer-promoter interactions are constrained, aiding the cell-type specificity of promoter function.

Lastly, I will present some disadvantageous aspects of the CRISPR/Cas9 technology. In addition to the widely discussed off-target mutagenesis, there are some other consequences of CRISPR/Cas9 which can cause problems in the laboratory. The introduction of specific mutations into the genome on one allele is frequently accompanied by deleterious indel mutations on the other allele. The resulting compound heterozygous genotypes can complicate downstream analysis. Furthermore, the persistent activity of CRISPR/Cas9 nucleases beyond the one-cell division leads to the frequently observed genetic mosaicism, where individual cells of a stem cell colony or embryo harbour different combinations of alleles. I will introduce some preliminary data demonstrating how we are trying to tackle these problems.
Reviewing the current EU regulatory environment for use of genome editing technology

Dennis Eriksson

Swedish University of Agricultural Science, Uppsala.

Since 1990, the European Union (EU) is implementing a specific regulatory environment for genetically modified organisms (GMOs) and their derived products. In the light of emerging techniques though, such as genome editing, this framework is getting increasingly ambiguous and difficult to interpret. The definition of a GMO in the EU is based on the use and stable incorporation of recombinant nucleic acids in a manner that cannot occur naturally. Mutagenesis is considered to result in GMO but exempt from the regulatory requirements. However, it is not further defined 1) what is considered natural, 2) what is mutagenesis, or 3) what is a recombinant nucleic acid. Several emerging techniques may apply a form of recombinant nucleic acids, but leaving only minor mutations in the resulting organism, and their regulatory status is therefore open for interpretation.

Various EU entities have contributed to the analysis of the regulatory status of these techniques, including a European Commission New Techniques Working Group, the European Food Safety Authority (EFSA), the European Parliament Research Service and others. Various cases, such as an ODM-modified rapeseed from the US-based company Cibus, a CRISPR-modified Arabidopsis for research purpose in Sweden, and a current case on mutagenesis in the Court of Justice of the European Union (CJEU), have triggered several EU member states to comment on the regulatory status of these techniques.

This presentation will outline the developments of various regulatory positions in the EU for emerging genome editing techniques and describe the difficulties in relation to the (lack of) definitions contained in the GMO regulatory framework. The speaker will also try to project some potential future policy developments in this area, inspired by recent developments in other countries and regions of the world.
As we approach the end of the first quarter-century of commercially available genetically engineered foods, the regulatory approach to such products has never been more chaotic and divisive.

There are two levels of division:

First, different countries have adopted significantly different regulatory approaches, creating serious trade and political conflicts between nations.

Second, within each nation, there are strong disagreements and contradictions in the regulatory approach to genetically engineered agricultural products.

The advent of more precise gene editing techniques creates the opportunity to adopt a more risk-based regulatory approach that can help to address the disharmony that exists at both the national and international levels. Using the United States as a case example, this presentation will discuss how gene editing is creating a window of opportunity for a more rational, risk-based approach to the regulation of agricultural biotechnology.

The presentation will also address how these changes within national regulatory systems provide promise for a more harmonized and risk-based international governance of gene-edited agricultural products.
Should genome-edited organisms be classed as GMOs? A UK perspective.

Louise Ball

UK Government Department for Environment, Food and Rural Affairs (DEFRA)

The UK’s GMO legislation has been transposed from EU legislation. England, Northern Ireland, Scotland and Wales each have their own versions and are responsible for implementation at a national level (e.g. in authorizing GM field trials). Authorizations for the commercial use of GMOs (e.g. commercial cultivation or importing GM animal feed) are dealt with by centralized EU processes in which the UK acts as one authority. The legislation aims to establish which organisms produced by modern biotechnology should be captured and the assessment and decision-making processes that follow if the legislation applies. This should be applied consistently across the EU.

However, the advent of so-called ‘new techniques’ such as genome-editing has highlighted different ways that the EU’s/UK’s definition of a GMO can be interpreted. To what extent the method used to produce an organism determines its regulatory status is of particular importance. The UK government’s intervention in a current European Court of Justice Case was based on the premise that the legislation was drafted to capture organisms produced by modern biotechnology that could not have been developed using traditional breeding techniques. This is approach is compatible with those adopted by many countries outside of the EU.

The EU Commission is reticent about amending the GMO legislation because of the political disagreement amongst its member states. This divergence of views makes it difficult to project the outcome of any such initiative. Post EU exit we have an opportunity to consider whether we would like to amend our regulatory approach/framework. In the first instance, the legislation we have will roll-over so that there is no step change. We believe that we can make the current system work more effectively by a more scientific interpretation of the definition and a proportionate case by case approach to assessing organisms that are captured by the GMO legislation – without compromising safety.
Gene Editing: A European cereal breeding perspective.

Christopher Burt

RAGT Seeds

For many years cereal breeders have utilised marker assisted selection of QTL and major genes. However, we now increasingly have knowledge of the underlying causal genes and ‘perfect’ markers to select for beneficial alleles. More recently an increased understanding of the underlying genetics and the availability of a TILLING resource in wheat has enabled breeders to identify mutants within known genes and utilised these to provide novel variation in their programmes. However, this requires time-consuming backcrossing into elite varieties to clean-up mutational loads and to transfer the target mutation into agronomically suitable backgrounds. Therefore, the ability to make targeted gene changes through CRISPR-Cas9 directly in elite varieties would be highly advantageous. Whilst plant breeders are now using the results from CRISPR-Cas9 experiments from academic research to inform their work it is not widely being used in crop variety development.

There are a number of technical, intellectual property, and regulatory issues to overcome before plant breeders will be able to utilise this technology.

- There are still a limited number of cloned genes to target. Of those that have been characterised, there is often little functional understanding of sufficient level to enable the use of gene modification rather than targeted knock-outs.
- Smaller plant breeding companies without internal expertise and facilities will need access to services or collaborations to generate the gene edits.
- There is a need for resolution on intellectual property ownership of the CRISPR-Cas9 technique itself. Whilst non-exclusive licenses for academic research are offered readily, breeding companies need clarification on the patent ownership issue to legally utilise the technology in variety development.
- Regulatory clarity is required from the European Union. A ruling is expected from the European Court of Justice in 2018 on whether CRISPR-cas9 is exempt from the GMO directive, as mutagenesis currently is. The seed industry takes the position that plant varieties developed from new breeding technologies should not be subject to different regulation if they are indistinguishable from varieties developed using traditional breeding methods, or could also have been obtained from natural processes.
Gene editing in *Physcomitrella patens*

**Fabien Nogue**

**INRA Centre de Versailles-Grignon, 78026 Versailles Cedex France**

We have already reported the successful use of the CRISPR-Cas9 gene editing technique in the nonvascular plant Physcomitrella patens for gene knock-out or gene knock-in (Collonnier et al., 2017). In this study we could observe that deletions involving local micro-homologies were a major source of mutations after the induction of a double strand break (DSB) through CRISPR-Cas9. We have now generalised this observation to DSBs induced through meganuclease, TALEN and CPF1. These deletions could be due, potentially, to the alternative-EJ DNA repair (alt-EJ) pathway. Indeed alt-EJ is an error prone pathway that uses sequence microhomology to repair DSB, and that is Ku 70/80 and Ligase IV independent. The mechanism behind this pathway, and its importance for DNA repair, are still not fully understood, moreover, most of the proteins involved in alt-EJ are still unknown.

Previous molecular and phylogenetic studies have proposed an atypical A-family polymerase, the polymerase Q (POLQ), also named POL¿, as a main factor involved in alt-EJ mediated DNA repair. We will present here recent data on the role of POLQ in DNA repair of double strand breaks in P. patens. We could show that POLQ is essential for alt-EJ repair of double strand breaks in P. patens and that POLQ-mediated repair of double strand breaks did not necessarily involves existing microhomologies. We also demonstrate that POLQ is essential to random integration of transgenes and a strong inhibitor of the Homology Directed Repair (HDR) pathway. This has a consequence in term of genetic engineering as in absence of POLQ the frequency of gene targeting (HDR mediated) is significantly increased and the number of clean two-sided HR-mediated insertions is enhanced. Thus, controlling POLQ activity in plants could be a useful strategy to increase gene knock-in efficiency and quality for plant breeding.
Nanomaterials for the delivery of gene-editing in plants

Heather M. Whitney, Cara Doyle, Keith Edwards, M. Carmen Galan

School of Biosciences, Bristol University, UK

The ability to transform plants has enabled both fundamental insights into plant biology through the modification of model species and revolutionised commercial agriculture. It is also central to future technologies such as plant synthetic biology and genome editing. However, a major bottleneck in taking full advantage of the potential of some technological advances has been that for most species transformation and/or plant regeneration from tissue culture is still slow, arduous, inefficient, species and variety specific.

We are investigating new mechanisms of delivering gene-editing machinery to plant cells using carbon-based synthesised nanomaterials. These nanomaterials are fluorescent so their movement through the plant can be monitored, and are functionalised to carry specific loads such as DNA.

I will discuss the problems and potential advantages of combining the two fast moving fields of gene-editing and nanotechnology.
Expression of CRISPR using Viral Vectors for Targeted Mutagenesis

Thomas Birkett, C. Payacan, C. Sari, S. Jackson

School of Life Sciences, Gibbet Hill, Warwick University.

t.birkett@warwick.ac.uk

Genome editing can be used to produce desired traits in crop plants. The CRISPR-Cas9 system can be designed to target a specific DNA sequence and cause targeted mutations in the genome. As an alternative to producing transgenic plant lines to express the CRISPR-Cas9 system, transient expression of CRISPR-Cas9 from non-integrating viral vectors can be used to express the Cas9 protein and sgRNAs systemically in plants.

In this project we aim to use virally-expressed Cas9 and sgRNAs to modify the Flowering Locus T (FT) gene in Nicotiana tabacum. FT plays a central role in the control of flowering in plants and mutations in the FT gene can delay flowering. We have created Potato Virus X (PVX) and Tobacco Rattle virus (TRV) vectors expressing a CRISPR-Cas9 cassette with sgRNAs designed to the N. tabacum FT4 gene, and demonstrated the systemic expression of the Cas9 mRNA, protein and the sgRNAs in virally-infected plants.

We are now investigating whether the expressed Cas9 and sgRNAs have caused mutations in the targeted FT sequences in infected plants. We will also analyse the F2 generation to see if mutations seen in the F1 generation are hereditable, and also to identify which F2 plants are virus-free.
Application of protoplast technology to CRISPR/Cas9: single protoplast isolation and protoplast regeneration in tobacco

Choun-Sea Lin¹, Chen-Tran Hsu¹, Ling-Hung Yang¹, Lan-Ying Lee², Jin-Yuan Fu¹, Qiao-Wei Cheng¹, Fu-Hui Wu¹, Chen-Ting Yu¹, Li-Jen Liao³, Stanton B. Gelvin², Ming-Che Shih¹

¹- Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan
²- Department of Biological Sciences, Purdue University, West Lafayette, Indiana, 47907-1392, USA
³- Institute of Life Science, National Kaohsiung Normal University, Kaohsiung, Taiwan

Plant protoplasts are important for Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR Associated Protein 9 (Cas9) mutagenesis. We developed a method to isolate single mutagenized Nicotiana tabacum protoplasts. Following transfection of protoplasts with constructs encoding SaCas9 and sgRNAs, target gene phytoene desaturase (NtPDS) DNA could be amplified and to determine mutagenesis efficiency.

We investigated *N. tabacum* protoplasts and derived regenerated plants for targeted mutagenesis. Genotyping of albino regenerants indicated that all four NtPDS alleles were mutated in amphidiploid tobacco, and no Cas9 DNA could be detected in most regenerated plants. Dwarfing, flowering time, and male fertility. The modular and orthogonal nature of these synthetic transcription factors should allow ready application to a wide variety of plant species.
The GA-biosynthesis pathway can be re-programmed in a model driven manner using hormone activated Cas9-based repressors (HACRs)

Alexander R Leydon, Arjun Khakhar, Andrew C Lemmex, Eric Klavins, Jennifer L Nemhauser

University of Washington, Washington State, USA

Maintaining food security requires the development of genetic tools to re-design morphological traits of crop plants for greater productivity in the face of climatic variability. Plant hormone circuitry is an obvious target for engineering as it regulates most developmental programs. The Nemhauser and Klavins labs have developed a set of synthetic and modular hormone activated Cas9-based repressors (HACRs) that respond to the phytohormones auxin, jasmonate and gibberellic acid (GA). Here, we will focus on how GA-HACRs can be used to reprogram development to improve yield-associated traits.

We have programmed GA-HACRs to target GA biosynthesis and signaling components to modulate the total output of the GA hormone-signaling pathway. To most effectively predict and calibrate the impact of GA-HACRs, we implemented a mathematical model of GA production and signaling with and without GA-HACRs. We demonstrate that GA-HACRs are sensitive to the endogenous pattern of bioactive GA in tissues with well-documented GA maxima.

We observed that targeting GA-HACRs to GA20 oxidase effectively reduces root length and delays flowering time, consistent with the predictions of the mathematical model. Tuning GA signaling by integrating GA-HACRs into the endogenous hormone pathway allows specific targeting of agriculturally relevant GA-responses such as dwarfing, flowering time, and male fertility. The modular and orthogonal nature of these synthetic transcription factors should allow ready application to a wide variety of plant species.
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Genome Targeting of Meiotic Recombination in plants

Salman Aloufi, Eugenio Sanchez-Moran

School of Biosciences, University of Birmingham, B15 2TT, UK

Sxx469@bham.ac.uk

DNA Double Strand Break (DSB) is one of the most serious damages for eukaryotic genomes. DSBs can be introduced by exogenous factors such as chemicals and ionizing radiation (gamma rays), or by endogenous factors such as free radicals and different enzymes (e.g.: Spo11 during meiosis). If DSBs are not properly repaired, they could lead to genomic instability or cell death. DSBs can be repaired by three different pathways, Non-Homologous End Joining (NHEJ), Homologous Recombination (HR), or Single Strand Annealing (SSA). NHEJ and SSA are error prone pathways whereas HR is error-free. HR uses a DNA template sequence (sister chromatid) to repair the DSB and thus, to maintain the genomic sequence.

HR is also the main pathway that processes meiotic DSBs catalyzed by Spo11 which would produce crossovers (COs) or non-crossovers (NCOs). During meiosis the template that is used is the homologous chromosome. At least one CO formation (the obligatory chiasma) is required for holding homologous chromosomes until they are segregated to opposite poles at anaphase I. The Arabidopsis DSB formation machinery involves several proteins: SPO11-1/SPO11-2 intermediate DSB formation by interacting with different accessory proteins (i.e.: MTOPVIB, PRD1, PRD2, PRD3). PRD2 is known to possess several motifs as in DNA-binding proteins, so it can have a role in promoting DSB formation after binding to the chromosomes.

Different mechanisms have been used to induce artificial DSBs in various organisms including plants. IR, such as X-rays and gamma-rays, has been used frequently as well as several chemicals like cisplatin or etoposide. Nevertheless, all these techniques create DSBs distributed randomly along the genome. Recently, Artificial Recombinase Endonucleases have been engineered to sequence-specific protein domains which have allowed producing DSBs in specific sequences in the genome. This has allowed to dream on Genome Targeting or Genome Editing in different species. Zinc Finger Nucleases (ZFNs) have been successfully used to induce DSBs in specific sequences in the genome.

In the present study, we have used genome specific ZFNs to induce DSBs during meiosis artificially in a specific position on the Arabidopsis genome (on TT4 and ADH1 loci). The analysis has been carried out in an Atspo11.2 null mutant background where not DSBs could be catalyzed during meiosis. Using an inducible system to express the ZFNs at different time points during meiosis has produced promising results showing that using this system will allow to target COs to an specific genome sequence. This study produces new insights into the manipulation of meiotic recombination in plants.

MitoCRISPR: Engineering Cas12a to target mitochondrial DNA

Zuriñe Antón, Holly Ford, Grace Mullally, Marc van der Kamp, Ian Collinson, Jon Lane, Mark Szczelkun

School of Biochemistry, University of Bristol, Bristol, BS8 1TD

zuri.anton@bristol.ac.uk

Mutations in mitochondrial DNA (mtDNA) lead to diseases with variable phenotypes. There is presently a lack of mitochondria-specific molecular biology tools, impeding understanding of mitochondrial genetics and disease. The mitoCRISPR project aims to re-engineer CRISPR machinery to allow reliable trafficking to human mitochondria, transport across the double membrane, and reconstitution of DNA-binding and cleavage activity within the matrix. This multidisciplinary synthetic biology project involves: (1) Re-engineering of CRISPR nucleases including use of alternative mitochondrial targeting sequences (MTSs) and smaller paralogues; (2) Re-engineering of gRNAs including addition of mitochondrial targeting aptamers and protein MTSs; (3) Expression of nucleases in cultured human cells and transfection of cells with purified nucleases and gRNAs; (4) Assessment of mtDNA cleavage activity in mitochondrial disease cell lines (MELAS cybrids). We have designed and re-engineered several versions of CRISPR nucleases including Streptococcus pyogenes (Spy) Cas9, Streptococcus aureus (Sa) Cas9, Acidaminococcus. sp. (As) Cas12a and Lachnospiraceae bacterium (Lb) Cas12a. Fluorescence microscopy, subcellular fractionation, immunoblotting and Seahorse XF Cell Mitochondria Stress Test have shown efficient mitochondrial targeting of Cas12a proteins with various N-terminal MTSs to healthy mitochondria in hTERT-immortalised human RPE1 cells and MELAS cybrids.

The Type V CRISPR enzyme LbCas12a is now our primary focus for development of this system. It traffics efficiently to mitochondria using the N-terminal MTS derived from Neurospora crassa ATP synthase subunit 9 (SU9), and has a smaller gRNA structure than the commonly used SpyCas9 CRISPR system.
Expression of CRISPR using Viral Vectors for Targeted Mutagenesis.

T. Birkett, C. Payacan, C. Sari, S. Jackson
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A CRISPR way to edit the honey fungus

Kathryn L. Ford, Finlay H. M. Bourquin

School of Biological Sciences, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TQ

The honey fungus (Armillaria sp.) is a devastating root disease pathogen affecting numerous fruit, nut and timber crops worldwide, causing up to 40% yield losses. It is also a major problem in gardens in the UK, coming top of the Royal Horticultural Society (RHS) Advisory Service pathogen enquiry list for the past 21 years.

Armillaria root disease is particularly difficult to manage due to its persistence in the soil, broad host range, phasing out of chemical controls and lack of understanding of the genetic factors controlling pathogenicity hampering development of novel management techniques. Unusually for a fungus, Armillaria is diploid, so usage of conventional genetic tools is problematic and no methods for analysis of gene function are currently available for this species.

A CRISPR-Cas9 editing system for *A. mellea* would provide such a method. Shown to be functional in several fungal species, CRISPR-Cas9 is still in its infancy for engineering of mushroom-forming fungi, with only three examples reported to date. We aim to develop a preliminary CRISPR-Cas9 system for gene editing in *A. mellea*, which if successful could then be deployed as a tool to understand the genetic basis of pathogenicity in this mushroom.

Work will be presented on the construction of CRISPR-Cas9 transformation vectors and the testing of vector functionality in a fast-growing model mushroom species Coprinopsis cinerea, which has an established CRISPR-Cas9 gene editing method.

Pinpointing TCTP role in pollen tube guidance with CRISPR-Cas9

Said Hafidh¹ and David Honys¹,²

¹- Institute of Experimental Botany, Laboratory of Pollen Biology, Prague Czech Republic
²- Department of Plant Experimental Biology, Faculty of Science, Charles University, Vinicná 5, 128 44, Prague 2, Czech Republic

In animals, Translationally controlled tumor protein (TCTP) is secreted via exosomes and function as an anti-apoptotic factor through misregulation of the mTOR pathway. In Arabidopsis, TCTP1 was shown to be essential for embryogenesis.

Recently, using pollen tube secretome assay and genetic analysis, we have reported that TCTP1 is secreted by the pollen tube and is a critical male-specific component in pollen tube guidance. tcp1 pollen tubes can poorly target ovules for fertilization and only the female gametophyte can efficiently transmit the tcp1 allele.

Here, we are using a CRISPR-Cas9 approach to edit TCTP domains as well as manipulate TCTP secretion to establish the exact mechanism of TCTP role in pollen tube guidance.

This project is supported by the Czech Science Foundation grants 17-23203S and 15-16050S
New genome editing strategies for manipulation of photosynthesis

Patricia Lopez Calcagno

School of Biological Sciences, University of Essex

Light-saturated photosynthesis in many crops is co-limited by Rubisco activity and the rate of regeneration of RuBP, the molecule that is used by Rubisco for accepting CO2. Modelling studies have suggested several points of co-limitation of RuBP supply (Zhu et al. 2013). To overcome these, a collection of transgenic plants overexpressing a suite of genes has been produced. Increased levels of SBPase, FBP Aldolase, H-protein, RieskeFeS, or the proteins responsible for the NPQ mechanism (Kromdijk et al., 2016; Lefebvre et al., 2005; Simkin et al., 2017a; Simkin et al., 2017b) or the introduction of foreign genes like SBPase/FBPase bifunctional enzyme, ictB and CytC6 (Chida et al., 2007; Kohler et al., 2017; Simkin et al., 2015) have proven successful strategies to increase photosynthesis and biomass in greenhouse and -for some- field conditions.

Nevertheless, our current approach requires the production of a large number of transgenic lines per manipulation which requires large amounts of space and efforts to characterize the phenotypes. In an effort to direct our transgenes into safe locations and hence decrease the amount of independent lines required for every manipulation and to move from away GM approaches, two genome editing strategies have been proposed.

1) Use of landing pads to direct all of our transgenes to a single safe location which promises to decrease the amount of lines needed per manipulation and simplify the comparisons between different manipulations.
2) Promoter edition to increase target gene expression levels, in potentially GM-free plants.

This poster will present details and progress on these strategies.

The BRACT crop transformation / genome editing platform

Tom Lawrenson, Mark Smedley, Sadiye Hayta, Alison Hinchliffe, Nicola Atkinson, Penny Hundleby, Wendy Harwood

The Crop Transformation Group, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH

The BRACT platform provides efficient Agrobacterium-mediated transformation and genome editing in a range of crops including wheat, barley, Brassica oleracea, Brassica napus, potato and tomato. RNA-guided Cas9-mediated targeted gene knockouts have been achieved in all species with efficiencies of over 80% in some cases. CRISPR / Cas9 technologies are making a major impact in many areas of research and offer new opportunities to develop improved crops through precise genome engineering.

With funding from BBSRC, we are currently providing a targeted gene knock-out resource to the UK research community. This will deliver knockouts of 60 target genes in wheat, barley, Brassica oleracea, Brassica napus and tomato. In addition, the group focuses on developing improved and new genome editing methodology in crops to expand the range of targeted modifications that can be made.
Establishment of transgene-free genome editing tools in rapeseed, potato and barley

Renate Lührs¹, Dirk Becker², Jörg Schondelmaier¹, Jon Falk¹

1- Saaten-Union-Biotec GmbH (SU Biotec), Hovedisser Straße 94, D-33818 Leopoldshöhe, Germany
2- Universität Hamburg, Biozentrum Klein Flottbek, Ohnhorststr. 18, D-22609 Hamburg, Germany

Targeted gene modification by the CRISPR/Cas9 system has been announced as an emerging genome editing tool in crop plants. However, this usually requires a plant transformation step with stable integration of the genes coding for cas9 and sgRNA into the plant genome (Arora & Narula 2017). Although the transgenes can later be eliminated by outcrossing, there are some disadvantages. (1) According to the European directives, progenies of transgenic plants are classified as GMO. (2) The constitutive expression of the transgenes, cas9 and sgRNA, might support off-targets and (3) the regeneration of chimeric plants carrying different mutations.

To overcome these issues, we are currently testing different approaches avoiding stable plant transformation. Using efficient protoplast regeneration systems, we started to establish a platform for targeted mutation via transient expression of CRISPR/Cas vectors. High throughput techniques which are routinely used for marker analysis at SU Biotec facilitate the targeted mutants screening for in a large population of calli and plants.

Additionally, we are developing methods for direct transfer of the Cas9 ribonucleoprotein complex into totipotent plant cells. We mainly focus on the protein transfer into rapeseed and barley microspores, because SU Biotec has an efficient and quite genotype independent regeneration platform which are routinely applied for double haploid production. We followed a modified approach of the research group of Eudes who reported that molecules can pass the microspore cell wall using cell penetrating peptides (Bilichak et al. 2015). We already showed that green fluorescence protein (as model) fused to cell penetrating peptides can be delivered into microspores of rapeseed and barley. Experiments to deliver the cas9 ribonucleoprotein complex are in progress.

Deciphering the Developmental Effects of HSFA1 Quadruple Knockout Mutant in Arabidopsis thaliana Generated Using CRISPR/Cas9

Irabonosi Obomigie

School of Biological Sciences, University of Essex

Arabidopsis thaliana clade A1 HSFs function as the master regulators of heat shock and abiotic stress response. When all four highly redundant homologous transcription factors (TFs) HSFA1a/b/d/e were knocked out by T-DNA insertions, the resulting quadruple mutant (QK) was impaired in physiological responses in relation to abiotic stress along with severe developmental defects. However, this original QK mutant was generated as a cross between 2 genotypes; Col-0 and Ws-0, which are morphologically different.

Due to the morphological differences in both accessions, a hybrid cross leads to developmental differences between individual hybrid progeny making it difficult to score developmental changes or effects of functional genes on developmental traits. Having been used successfully in generating double stranded breaks in desired regions in recent times, the CRISPR/Cas9 system was employed to knockout the remaining functional member of the clade A1, HSFA1a, from a homozygous triple mutant (bdeKO) in a single background (Col-0) due to unavailability of a T-DNA insertional mutant in Col-0.

Using fluorescence technology and high resolution melting analysis for selection, a positive Cas9-free quadruple knockout mutant (QK2) in a single background was generated. Two independent QK2 plants with different mutations (-10/+1 bp) were generated both having similar developmental effects however, developmental similarity was restricted to plant size when compared to QK strongly supporting the reason for this study. Some QK2 mutants also possessed pointed cotyledons compared to wildtype but the same leaf number suggesting the HSFA1s might function in plant cell expansion. Physiological responses to heat and high light as well as the transcriptomic profile of the QK2 compared to wildtype is under investigation.


Stomatal dysfunction in a BEACH protein mutant

Ashley Pridgeon

School of Biosciences, University of Bristol

Stomata are microscopic pores found on the surface of leaves that function to regulate gas exchange and water loss between the plant and its immediate environment. Each stoma is composed of two guard cells that are able to swell and deflate in response to various signals, regulating the aperture of the stomatal pore. The contribution of ion transport across guard cell vacuolar and plasma membranes is well known to be the key driving force eliciting the changes in pore size. However, the process of membrane trafficking and how this affects stomatal movement is less understood.

In this study, we look at Arabidopsis mutants within a member of an ancient family of conserved eukaryotic proteins known as BEACH domain containing proteins. This protein family have been linked to membrane trafficking processes in many eukaryotic species from plants to humans. Here, we show mutants within the gene, TPCR, display a stomatal phenotype where the pores are selectively dysfunctional in their responses to Ca²⁺⁺ and CO₂, while displaying a normal closure response to the drought hormone ABA.

Multiplex mutagenesis of four clustered CrRLK1L with CRISPR/Cas9 exposes their growth regulatory roles in response to metal ions

Julia Richter¹, James Matthew Watson²,³, Peter Stasnik¹, Vera Schoft², Marie-Theres Hauser¹

1- Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences (BOKU), Muthgasse 18, 1190 Vienna, Austria
2- Vienna Biocenter Core Facilities GmbH (VBCF), Dr. Bohrgasse 3, 1030 Vienna, Austria
3- Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna BioCenter (VBC), Dr. Bohrgasse 3, 1030 Vienna, Austria

Resolving functions of closely linked genes is challenging or nearly impossible with classical genetic tools. Four members of the Catharanthus roseus receptor-like kinase 1-like (CrRLK1L) family are clustered on Arabidopsis chromosome five. To resolve the potentially redundant functions of this subclass of CrRLK1Ls named MEDOS1 to 4 (MDS1 to 4), we generated a single CRISPR/Cas9 transformation vector using a Golden Gate based cloning system to target all four genes simultaneously.

We introduce single mutations within and deletions between MDS genes as well as knock-outs of the whole 11 kb gene cluster. The large MDS cluster deletion was inherited in up to 25% of plants lacking the CRISPR/Cas9 construct in the T2 generation. In contrast to described phenotypes of already characterized CrRLK1L mutants, quadruple mds knock-outs were fully fertile, developed normal root hairs and trichomes and responded to pharmacological inhibition of cellulose biosynthesis similar to wildtype.

Recently, we demonstrated the role of four CrRLK1L in growth adaptation to metal ion stress. Here we show the redundant involvement of MDS genes in response to Ni²⁺ during hypocotyl elongation and to Cd²⁺ and Zn²⁺ during root growth. Our finding supports the model of an organ specific network of positively and negatively acting CrRLK1Ls.
**Poster Abstract 12**

**Identification of development-regulated microRNA/target modules in *Arabidopsis thaliana* roots**

**Camila Rodríguez**, Gabriela Saavedra, Luis Larraín, Elena A. Vidal  
Centro de Genómica y Bioinformática, Facultad de Ciencias, Universidad Mayor  
camila.rodriguez.monroy@gmail.com

The plant root system senses, explores and uptakes nutrients from the soils. At a given time, root system architecture (RSA) is the result of the integration of external cues experienced by the plant into intrinsic developmental programs. Thus, plasticity of RSA is a dynamic process that changes during plant life cycle according to specific plant requirements to optimize growth in heterogenous and changing environments. Despite the importance of roots for plant growth and productivity, little is known on root plasticity regulators and their impact during different stages of plant development. This is of paramount importance for developing biotechnological applications that target the improvement of root systems for growth in stress conditions such as limiting nutritional conditions. Post-transcriptional gene silencing by small RNAs (sRNAs) is a key determinant of plant developmental processes.

To date, the patterns of expression of some sRNAs have been studied in detail during Arabidopsis development in shoot tissue and have been shown to be crucial for timing of phase change. Although root-expressed sRNAs controlling primary and lateral root growth have been identified, their role during specific stages of plant development, the gene regulatory networks (GRNs) that they control, or their role in adaptation to environmental conditions have not yet been identified.

In this work, we predicted Arabidopsis microRNA targets using bioinformatics programs and experimental data from available degradome-seq analyses and we analyzed their developmental expression patterns using root transcriptomics datasets. We were able to identify and experimentally confirm diverse development-regulated microRNA/TARGET modules in roots, including miR156/SPL and miR172/AP2 modules, whose temporal expression in shoots is known to control the timing of phase changes. We are analyzing the role of these modules on root development and response to limiting macronutrient availability, as well as identifying the GRNs that they control in roots.

Acknowledgements: FONDECYT #1170926, Proyecto #OI101040-Universidad Mayor

**Poster Abstract 13**

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camila.rodriguez.monroy@gmail.com

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## Meeting Delegates

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<tr>
<td>Chidi Afamefule</td>
<td>MPIPZ Cologne</td>
<td><a href="mailto:afamefule@mpipz.mpg.de">afamefule@mpipz.mpg.de</a></td>
</tr>
<tr>
<td>Salmon Aloufi</td>
<td>University of Birmingham</td>
<td><a href="mailto:abuayad87@gmail.com">abuayad87@gmail.com</a></td>
</tr>
<tr>
<td>Johanna Astrand</td>
<td>University of Nottingham</td>
<td><a href="mailto:johanna.astrand@nottingham.ac.uk">johanna.astrand@nottingham.ac.uk</a></td>
</tr>
<tr>
<td>Louise Ball</td>
<td>DEFRA</td>
<td><a href="mailto:louise.ball@DEFRA.GSI.GOV.UK">louise.ball@DEFRA.GSI.GOV.UK</a></td>
</tr>
<tr>
<td>Christopher Bell</td>
<td>University of Leeds</td>
<td><a href="mailto:bscb@leeds.ac.uk">bscb@leeds.ac.uk</a></td>
</tr>
<tr>
<td>Thomas Birkett</td>
<td>University of Warwick</td>
<td><a href="mailto:t.birkett@warwick.ac.uk">t.birkett@warwick.ac.uk</a></td>
</tr>
<tr>
<td>Amanda Burridge</td>
<td>University of Bristol</td>
<td><a href="mailto:amanda.burridge@bristol.ac.uk">amanda.burridge@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Baptiste Castel</td>
<td>The Sainsbury Lab, Norwich</td>
<td><a href="mailto:baptiste.castel@tsl.ac.uk">baptiste.castel@tsl.ac.uk</a></td>
</tr>
<tr>
<td>David Coronado</td>
<td>University of Bristol</td>
<td><a href="mailto:david.cuituncoronado@bristol.ac.uk">david.cuituncoronado@bristol.ac.uk</a></td>
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<tr>
<td>Alice Darbyshire</td>
<td>University of Warwick</td>
<td><a href="mailto:a.darbyshire94@gmail.com">a.darbyshire94@gmail.com</a></td>
</tr>
<tr>
<td>Elsa Dell’Aglio</td>
<td>University of Bristol</td>
<td><a href="mailto:elsa.dellaglio@gmail.com">elsa.dellaglio@gmail.com</a></td>
</tr>
<tr>
<td>Keith Edwards</td>
<td>University of Bristol</td>
<td><a href="mailto:k.j.edwards@bristol.ac.uk">k.j.edwards@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Dennis Eriksson</td>
<td>Swedish University of Agricultural Sciences</td>
<td><a href="mailto:Dennis.Eriksson@slu.se">Dennis.Eriksson@slu.se</a></td>
</tr>
<tr>
<td>Kathryn Ford</td>
<td>University of Bristol</td>
<td><a href="mailto:kathryn.ford@bristol.ac.uk">kathryn.ford@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Shannah Gates</td>
<td>Royal Holloway, University of London</td>
<td><a href="mailto:shannah.gates.2016@live.rhul.ac.uk">shannah.gates.2016@live.rhul.ac.uk</a></td>
</tr>
<tr>
<td>Murray Grant</td>
<td>University of Warwick</td>
<td><a href="mailto:M.R.Grant@exeter.ac.uk">M.R.Grant@exeter.ac.uk</a></td>
</tr>
<tr>
<td>Mona Alboushi</td>
<td>University of Bristol</td>
<td><a href="mailto:ma166.50@bristol.ac.uk">ma166.50@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Zuri Anton</td>
<td>University of Bristol</td>
<td><a href="mailto:zuri.anton@bristol.ac.uk">zuri.anton@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Sylvain Aubry</td>
<td>University of Zurich</td>
<td><a href="mailto:sylvain.aubry@blw.admin.ch">sylvain.aubry@blw.admin.ch</a></td>
</tr>
<tr>
<td>Fiona Belloni</td>
<td>University of Bristol</td>
<td><a href="mailto:fiona.belloni@bristol.ac.uk">fiona.belloni@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Sara Ben-Khaled</td>
<td>British American Tobacco</td>
<td><a href="mailto:sara_ben_khaled@bat.com">sara_ben_khaled@bat.com</a></td>
</tr>
<tr>
<td>Emily Breeze</td>
<td>University of Warwick</td>
<td><a href="mailto:emily.breeze@warwick.ac.uk">emily.breeze@warwick.ac.uk</a></td>
</tr>
<tr>
<td>Chris Burt</td>
<td>RAGT Seeds</td>
<td><a href="mailto:CBurt@ragt.fr">CBurt@ragt.fr</a></td>
</tr>
<tr>
<td>Yi Chen</td>
<td>John Innes Centre</td>
<td><a href="mailto:yi.chen@jic.ac.uk">yi.chen@jic.ac.uk</a></td>
</tr>
<tr>
<td>Alex Coulson</td>
<td>University of Bristol</td>
<td><a href="mailto:alex.coulson@bristol.ac.uk">alex.coulson@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Ben Davies</td>
<td>University of Oxford</td>
<td><a href="mailto:ben.davies@well.ox.ac.uk">ben.davies@well.ox.ac.uk</a></td>
</tr>
<tr>
<td>Eva Karina Díaz Sánchez</td>
<td>Royal Holloway, University of London</td>
<td><a href="mailto:eva.diazsanchez.2018@live.thul.ac.uk">eva.diazsanchez.2018@live.thul.ac.uk</a></td>
</tr>
<tr>
<td>Beth Eldridge</td>
<td>University of Bristol</td>
<td><a href="mailto:bethany.eldridge@bristol.ac.uk">bethany.eldridge@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Jose Fernandez</td>
<td>University of Nottingham</td>
<td><a href="mailto:jose.fernandez@nottingham.ac.uk">jose.fernandez@nottingham.ac.uk</a></td>
</tr>
<tr>
<td>Holly Ford</td>
<td>University of Bristol</td>
<td><a href="mailto:h.ford@bristol.ac.uk">h.ford@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Lila Grandgeorge</td>
<td>The Sainsbury Lab, Norwich</td>
<td>lila <a href="mailto:grandi@nbi.ac.uk">grandi@nbi.ac.uk</a></td>
</tr>
<tr>
<td>Said Hafidh</td>
<td>Institute of Experimental Botany, Prague</td>
<td><a href="mailto:hafidh@ueb-cas.cz">hafidh@ueb-cas.cz</a></td>
</tr>
<tr>
<td>Helen Harper</td>
<td>University of Bristol</td>
<td><a href="mailto:helen.harper@bristol.ac.uk">helen.harper@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Miaoyuan Hua</td>
<td>University of Nottingham</td>
<td><a href="mailto:sbxnh5@nottingham.ac.uk">sbxnh5@nottingham.ac.uk</a></td>
</tr>
<tr>
<td>Luca Ilping</td>
<td>Warwick University</td>
<td><a href="mailto:lilling@warwick.ac.uk">lilling@warwick.ac.uk</a></td>
</tr>
<tr>
<td>Stefan Jansson</td>
<td>Umea University</td>
<td><a href="mailto:stefan.jansson@umu.se">stefan.jansson@umu.se</a></td>
</tr>
<tr>
<td>Claire Kamei</td>
<td>Max Planck Institute, Cologne</td>
<td><a href="mailto:kamei@mpipz.mpg.de">kamei@mpipz.mpg.de</a></td>
</tr>
<tr>
<td>Sungyong Kim</td>
<td>Swedish University of Agricultural Sciences</td>
<td><a href="mailto:sung.yong.kim@slu.se">sung.yong.kim@slu.se</a></td>
</tr>
<tr>
<td>Suneetha Kota</td>
<td>University of York</td>
<td><a href="mailto:suneetha.kota@york.ac.uk">suneetha.kota@york.ac.uk</a></td>
</tr>
<tr>
<td>Alex Leydon</td>
<td>University of Washington</td>
<td><a href="mailto:alex.leydon@washington.edu">alex.leydon@washington.edu</a></td>
</tr>
<tr>
<td>Choun-Sea Lin</td>
<td>Sinica University, Taiwan</td>
<td><a href="mailto:cslin99@gate.sinica.edu.tw">cslin99@gate.sinica.edu.tw</a></td>
</tr>
<tr>
<td>Patricia Lopez</td>
<td>University of Essex</td>
<td><a href="mailto:pepeple@exesx.ac.uk">pepeple@exesx.ac.uk</a></td>
</tr>
<tr>
<td>Gary Marchant</td>
<td>Arizona State University</td>
<td><a href="mailto:gary.marchant@asu.edu">gary.marchant@asu.edu</a></td>
</tr>
<tr>
<td>Alistair McCormick</td>
<td>University of Edinburgh</td>
<td><a href="mailto:alistair.mccormick@ed.ac.uk">alistair.mccormick@ed.ac.uk</a></td>
</tr>
<tr>
<td>Guillermia Mendiondo</td>
<td>University of Nottingham</td>
<td><a href="mailto:guillermia.mendiondo@nottingham.ac.uk">guillermia.mendiondo@nottingham.ac.uk</a></td>
</tr>
<tr>
<td>Rhian Morgan</td>
<td>Wales Gene Park, Cardiff University</td>
<td><a href="mailto:MorganRR1@cardiff.ac.uk">MorganRR1@cardiff.ac.uk</a></td>
</tr>
<tr>
<td>Vladimir Nekrasov</td>
<td>Rothamsted Research</td>
<td><a href="mailto:vladimir.nekrasov@rothamsted.ac.uk">vladimir.nekrasov@rothamsted.ac.uk</a></td>
</tr>
<tr>
<td>Fabien Nogue</td>
<td>INRA Versailles</td>
<td><a href="mailto:Fabien.Nogue@versailles.inra.fr">Fabien.Nogue@versailles.inra.fr</a></td>
</tr>
<tr>
<td>Jill Harrison</td>
<td>University of Bristol</td>
<td><a href="mailto:jill.harrison@bristol.ac.uk">jill.harrison@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Lucy Hyde</td>
<td>University of Bristol</td>
<td><a href="mailto:lucy.hyde2@gmail.com">lucy.hyde2@gmail.com</a></td>
</tr>
<tr>
<td>Stephen Jackson</td>
<td>University of Warwick</td>
<td><a href="mailto:stephen.jackson@warwick.ac.uk">stephen.jackson@warwick.ac.uk</a></td>
</tr>
<tr>
<td>Glynwynder Jones</td>
<td>University of Bristol</td>
<td><a href="mailto:gj14238@bristol.ac.uk">gj14238@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Maxim Kapralov</td>
<td>Newcastle University</td>
<td><a href="mailto:maxim.kapralov@ncl.ac.uk">maxim.kapralov@ncl.ac.uk</a></td>
</tr>
<tr>
<td>Marie Komrsova</td>
<td>University of Nottingham</td>
<td><a href="mailto:komrsova@nottingham.ac.uk">komrsova@nottingham.ac.uk</a></td>
</tr>
<tr>
<td>Tom Lawsonren</td>
<td>John Innes Centre</td>
<td><a href="mailto:tom.lawrenson@jic.ac.uk">tom.lawrenson@jic.ac.uk</a></td>
</tr>
<tr>
<td>Catherine Lilley</td>
<td>University of Leeds</td>
<td><a href="mailto:C.J.Lilley@leeds.ac.uk">C.J.Lilley@leeds.ac.uk</a></td>
</tr>
<tr>
<td>Andrew Lloyd</td>
<td>Aberystwyth University</td>
<td><a href="mailto:andl50@aber.ac.uk">andl50@aber.ac.uk</a></td>
</tr>
<tr>
<td>Renate Luehrs</td>
<td>Saaten-Union Biotec</td>
<td><a href="mailto:luehrs@saaten-union-biotec.com">luehrs@saaten-union-biotec.com</a></td>
</tr>
<tr>
<td>Sarah Matar</td>
<td>Liel University</td>
<td><a href="mailto:s.matar@plantbreeding.uni-kiel.de">s.matar@plantbreeding.uni-kiel.de</a></td>
</tr>
<tr>
<td>Michalea McGinn</td>
<td>Illinois State University</td>
<td><a href="mailto:michaelamcginnlopes@gmail.com">michaelamcginnlopes@gmail.com</a></td>
</tr>
<tr>
<td>Jackline Moraa Getuba</td>
<td>Premier Seeds</td>
<td><a href="mailto:jacklymoraa72@gmail.com">jacklymoraa72@gmail.com</a></td>
</tr>
<tr>
<td>Rose Murray</td>
<td>University of Bristol</td>
<td><a href="mailto:rose.murray@bristol.ac.uk">rose.murray@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Lauren Nicol</td>
<td>Vrije Universiteit Amsterdam</td>
<td><a href="mailto:laurenicol@outlook.com">laurenicol@outlook.com</a></td>
</tr>
<tr>
<td>Irabonosi Obomighie</td>
<td>University of Essex</td>
<td><a href="mailto:osyrina2@yahoo.com">osyrina2@yahoo.com</a></td>
</tr>
<tr>
<td>Jacky Moraa Getuba</td>
<td>Premier Seeds</td>
<td><a href="mailto:jacklymoraa72@gmail.com">jacklymoraa72@gmail.com</a></td>
</tr>
<tr>
<td>Rose Murray</td>
<td>University of Bristol</td>
<td><a href="mailto:rose.murray@bristol.ac.uk">rose.murray@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Lauren Nicol</td>
<td>Vrije Universiteit Amsterdam</td>
<td><a href="mailto:laurenicol@outlook.com">laurenicol@outlook.com</a></td>
</tr>
</tbody>
</table>
### Meeting Delegates

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geraint Parry</td>
<td>GARNet, Cardiff University</td>
<td><a href="mailto:geraint@garnetcommunity.org.uk">geraint@garnetcommunity.org.uk</a></td>
</tr>
<tr>
<td>Ashley Pridgeon</td>
<td>University of Bristol</td>
<td><a href="mailto:ashley.prigeon@bristol.ac.uk">ashley.prigeon@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Yiping Qi</td>
<td>University of Maryland</td>
<td><a href="mailto:yiping@umd.edu">yiping@umd.edu</a></td>
</tr>
<tr>
<td>Julia Richter</td>
<td>BOKU, Vienna</td>
<td><a href="mailto:julia.richter@boku.ac.at">julia.richter@boku.ac.at</a></td>
</tr>
<tr>
<td>Camila Rodriguez</td>
<td>Universidad Mayor, Santiago, Chile</td>
<td><a href="mailto:camila.rodriguez.monroy@gmail.com">camila.rodriguez.monroy@gmail.com</a></td>
</tr>
<tr>
<td>Gunjan Sharma</td>
<td>University of Nottingham</td>
<td><a href="mailto:gunjan.sharma@nottingham.ac.uk">gunjan.sharma@nottingham.ac.uk</a></td>
</tr>
<tr>
<td>Charles Spillane</td>
<td>University of Galway</td>
<td><a href="mailto:charles.spillane@nuigalway.ie">charles.spillane@nuigalway.ie</a></td>
</tr>
<tr>
<td>Tijmen van Butselaar</td>
<td>Utrecht University</td>
<td><a href="mailto:t.vanbutselaar@uu.nl">t.vanbutselaar@uu.nl</a></td>
</tr>
<tr>
<td>Andreas Weber</td>
<td>University of Dusseldorf</td>
<td><a href="mailto:andreas.weber@uni-duesseldorf.de">andreas.weber@uni-duesseldorf.de</a></td>
</tr>
<tr>
<td>Heather Whitney</td>
<td>University of Bristol</td>
<td><a href="mailto:heather.whitney@bristol.ac.uk">heather.whitney@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Sam Workman</td>
<td>University of Bristol</td>
<td><a href="mailto:sw13877@bristol.ac.uk">sw13877@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Claudia Payacan</td>
<td>University of Warwick</td>
<td><a href="mailto:claudia.payacan@gmail.com">claudia.payacan@gmail.com</a></td>
</tr>
<tr>
<td>Ian Prosser</td>
<td>University of Bristol</td>
<td><a href="mailto:ian.prosser@bristol.ac.uk">ian.prosser@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Dora Ramirez</td>
<td>University of Bristol</td>
<td><a href="mailto:dora.canoramirez@bristol.ac.uk">dora.canoramirez@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Jordan Robson</td>
<td>University of Nottingham</td>
<td><a href="mailto:sbjr13@exmail.nottingham.ac.uk">sbjr13@exmail.nottingham.ac.uk</a></td>
</tr>
<tr>
<td>Chamikla Rukshani-Thilakratane</td>
<td>University of South Wales</td>
<td><a href="mailto:15090124@students.southwales.ac.uk">15090124@students.southwales.ac.uk</a></td>
</tr>
<tr>
<td>Daniel Shaw</td>
<td>University of Bristol</td>
<td><a href="mailto:daniel.shaw@bristol.ac.uk">daniel.shaw@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Partha Talukdar</td>
<td>University of Aberdeen</td>
<td><a href="mailto:partha.omics@gmail.com">partha.omics@gmail.com</a></td>
</tr>
<tr>
<td>Emma Wallington</td>
<td>NIAB</td>
<td><a href="mailto:emma.wallington@niab.com">emma.wallington@niab.com</a></td>
</tr>
<tr>
<td>Mike Whitfield</td>
<td>New Phytologist Trust</td>
<td><a href="mailto:m.whitfield@lancaster.ac.uk">m.whitfield@lancaster.ac.uk</a></td>
</tr>
<tr>
<td>Mark Winfield</td>
<td>University of Bristol</td>
<td><a href="mailto:mark.winfield@bristol.ac.uk">mark.winfield@bristol.ac.uk</a></td>
</tr>
<tr>
<td>Lianyu Yuan</td>
<td>John Innes Centre</td>
<td><a href="mailto:lianyu.yuan@jic.ac.uk">lianyu.yuan@jic.ac.uk</a></td>
</tr>
</tbody>
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