



Accepted applications in the Community Resource for Wheat and Rice Transformation Project (2018-2023)

Exploring how temperature dependent development genes can be used to slow the reproductive transition in spring wheat

Dr Laura Dixon, University of Leeds

Spring wheat is widely grown across the world and offers the advantage of a short crop lifecycle which can fit between unfavourable growing seasons or other crops in fast rotation. However, this fast lifecycle significantly reduces the yield of spring wheat compared to winter wheat. This project will test if delaying growth during the vegetative to floral transition can reduce this yield loss through enabling the production of additional spikelets.

Engineering micronutrient content in wheat

Dr Philippa Borrill, University of Birmingham

Wheat, like many other staple cereals, contains low levels of the essential micronutrients iron and zinc. A gene has been identified in rice that increases iron and zinc content in the grain which could increase micronutrient intake in the human diet and therefore improve human health. Here we propose to test whether this gene could also be effective in wheat to improve iron and zinc content.

Improving abiotic stress tolerance in wheat using a transcription factor identified in rice

Dr Philippa Borrill, University of Birmingham

Abiotic stresses such as drought and salinity are major constraints on global crop production. A transcription factor has been identified in rice that improves tolerance to drought, salinity and cold stresses but we do not know if this gene could also be used to increase wheat stress tolerance. We propose to overexpress the wheat ortholog of this transcription factor to test whether overexpression leads to an increase in tolerance to drought, salinity and cold stress in wheat.

Zn sensing in wheat- a strategy for increasing micronutrient efficiency

Dr Lorraine Williams, University of Southampton

Cereals yield less and have lower nutritional quality when grown in soils where zinc (Zn) availability is low.

Deficiency of Zn on cultivated soils is a worldwide problem so developing plants that can maintain growth and yield under low soil Zn without the input of costly fertilisers would have clear benefits for sustainable agriculture. The Zn-sensing F-group bZIP transcription factor, HvbZIP1, will be expressed to test for improved Zn-deficiency tolerance and more widely for resistance to oxidative stress.

Callose enriched wheat, an innovative way to improve health and dietary value for human and livestock consumption

Prof. Yrjö Helariutta, Sainsbury Laboratory University of Cambridge

Straw is an important agricultural side-product of Wheat production, currently used for bioenergy production and livestock feed. We demonstrated that increasing callose (a Beta 1-3 glucan) content in poplar wood leads to increased deconstruction properties into fermentable sugars. On another hand, beta glucans have been shown to be beneficial when supplementing human diet and livestock feed. By increasing the Beta 1-3 glucans of wheat grain and straw, we aim to improve wheat biomass for versatile outcomes, from food to bioenergy.

Exploit silenced genes for wheat breeding

Dr Marco Catoni, University of Birmingham

The aim of this project is to build a wheat line transformed with a transcriptional silenced reporter gene. This transgenic reporter line will be used to determine conditions able to efficiently suppress transcriptional silencing in wheat. The release of transcriptional silencing can potentially activate genes and transposable elements not normally expressed in natural conditions, generating new stable traits, and the exploitation of this genetic and epigenetic variation in breeding program can potentially contribute to develop better varieties.

Developing auxin reporters in wheat

Dr Tom Bennett, Dr Stefan Kepinski and Dr Laura Dixon, University of Leeds

We aim to create a wheat version of the widely used auxin-responsive DII-VENUS reporter gene, along with the non-auxin responsive mDII-VENUS control gene, as tools to understand auxin response and distribution during wheat development. This will be a valuable tool for the emerging wheat development community, and will help to understand how fine-tuning auxin responses in elite wheat varieties could lead to yield increases (e.g. by increased spikelet formation) or increased resilience (e.g. steeper and deeper root systems).

Manipulating CHLORAD in wheat: Altering expression of the plastid retrotranslocon SP2 in order to develop novel crop improvement strategies

Prof. Paul Jarvis, University of Oxford

Recent work in *Arabidopsis* led to the discovery of a novel ubiquitin-dependent pathway of plastid proteostasis, which we termed CHLORAD (for chloroplast-associated protein degradation) (Ling et al., *Science*, 2019). Manipulation of the CHLORAD pathway in *Arabidopsis* leads to traits that are of interest in the development of new crop improvement strategies (e.g., improved stress tolerance, delayed senescence). This project will develop proof-of-principle in this regard by manipulating in wheat the expression of SP2, a central component of the CHLORAD pathway.

Discovering the role of Jasmonic acid in fungal disease resistance in wheat

Prof. Ari Sadanandom, University of Durham

Fungal diseases account for the greatest losses to wheat yield globally. The phytohormone Jasmonic acid (JA) has essential roles in growth and immunity. This project aims to reveal a new facet to JA signalling in wheat. We have identified a new genetic regulator of JA responses in wheat called Septoria Resistant 1 (TaSeR1) that facilitates resistance against the fungal pathogen *Zymoseptoria tritici*. We aim to gene-silence TaSeR1 to ascertain how it mediates fungal disease resistance in wheat.

Defining the role of exoribonucleases in drought tolerance

Dr Matt Jones, University of Glasgow

One of the first metabolic consequences of osmotic stress is the induction of 5'-PhosphoAdenosine 3'-Phosphate (PAP), which activates a retrograde signalling cascade that promotes ABA-induced signalling pathways- in part by inactivating a family of exoribonucleases. Constitutive production of PAP

promotes drought tolerance but has negative consequences on plant growth. We will knockdown expression of candidate exoribonucleases (downstream of PAP accumulation) to promote drought tolerance while minimising the adverse effects on growth.

Generating knockout alleles in a wheat male-germline regulator

Prof. David Twell, University of Leicester

The transcription factor DUO POLLEN1 (DUO1) ensures plant fertility by controlling sperm cell development in pollen of *Arabidopsis thaliana*. The knockout of DUO1 function in wheat would allow gamete differentiation and germline regulatory networks to be studied for the first time in a UK crop. These transgenics would also assist in the assembly of a broader molecular "toolbox" of regulatory components relevant to hybrid wheat research in collaboration with NIAB.

Generating a wheat TaDDM1 knockdown line to explore the potential of cytosine DNA hypomethylation to improve bread wheat

Dr Alison Bentley, NIAB and Dr Natasha Yelina, University of Cambridge

Cytosine DNA methylation is a regulatory epigenetic mark that contributes to genome stability and controls gene expression. Several protein factors, including DDM1, are required for cytosine DNA methylation maintenance. Cytosine DNA hypomethylated *ddm1* mutants in *Arabidopsis* show an altered landscape of meiotic recombination and compromised heterosis, or hybrid vigour. We propose to generate wheat *taddm1* mutants via CRISPR/Cas9 gene editing in order to study the effect of cytosine DNA hypomethylation on meiotic recombination and hybrid vigour in bread wheat.

Genome editing of tandemly duplicated candidate genes at the wheat Snn3-B1 locus controlling sensitivity to the effector Tox3

Dr James Cockram, NIAB

Stagonospora nodorum blotch (SNB) in wheat is caused by the fungal pathogen *Parastagonospora nodorum*. SNB resistance is underpinned by host response to protein effectors secreted by the pathogen. We used *P. nodorum* effector Tox3 to fine-map the corresponding wheat sensitivity locus, Snn3-B1. Overlaying RNAseq data (2 genotypes, 2 treatments, 5 time points, 3 biological reps per genotype/treatment/timepoint combination) and exploitation of the recently released wheat genome

assembly, we have identified a strong Snn3-B1 candidate for functional validation via CRISPR/Cas9.

Functional characterisation of a putative gene involved in parthenogenesis in the grass *Eragrostis curvula*

Prof. Mario Caccamo, NIAB

The forage grass *Eragrostis curvula* is an ideal model to study asexual reproduction by seed (apomixis). Using comparative genomics tools we have identified four genomic regions associated with this trait including a putative novel gene. Initial expression analysis supports the hypothesis for a role of this gene in the ovule development during parthenogenesis. We propose to transform this gene into rice with the aim of assessing its role in modulating the reproductive pathway in grasses.

Simultaneous improvement of drought tolerance and photosynthetic efficiency in *Oryza sativa*

Prof. Julie Gray, University of Sheffield

Improving agricultural productivity under future climates will require the combination of efficient photosynthesis and stress tolerance. This project will use recent findings to improve carbon assimilation and reduce water requirements in rice. The Calvin-Benson cycle will be enhanced by over-expressing fructose-1,6-bisphosphate adolase, and this trait will be introduced into our existing rice lines that have reduced stomatal densities. Combining these two traits will lead to improvements in water-use and photosynthetic rates, and thus more “crop per drop”.

Reducing rice overheating by increasing stomatal density

Dr Robert Caine, The University of Sheffield

Temperature increases are already putting rice yields under serious threat, and this is only set to worsen. By over-expressing a variant of *ICE1/SCRM (SCRM)* gene; *SCRM-d*, we will increase the number of stomata on rice leaves which should lead to an increase in transpirational cooling. Over-expression of the native *SCRM* gene improves yield in plants that have been droughted, and so it will also be interesting to see whether *SCRM-d* overexpressing plants retain some of this original *SCRM* functionality.

Engineering rice for methane detoxification

Dr Verena Kriechbaumer, Oxford Brookes University

Rice plants act as a chimney for the transfer of methane from the soil to the atmosphere. Fluxes of methane through plants in total are estimated to account for 10 to 40 % of annual global emissions. Hence plants are a valuable point of action for methane detoxification processes. The bacterial

particulate methane monooxygenase (pMMO) is the predominant methane oxidation catalyst in nature. We aim to engineer plants to produce the pMMO enzyme complex for methane detoxification.

Increasing seed size in rice

Dr Smita Kurup, Rothamsted Research

With rising population and diminishing agricultural land, it is increasingly urgent to improve crop yields. Increasing seed size and number in seed crop species are important routes to achieving this goal and improving food security. Previous work within my group and my collaborator Professor Scott's laboratory have shown that loss-of function mutations in the transcription factor AUXIN RESPONSE FACTOR 2 in Arabidopsis results in larger seed. We intend to investigate the consequences of loss of function of this gene in rice.

Manipulating guard cell sensitivity to blue light to improve productivity in wheat

Prof. Tracy Lawson, University of Essex

As stomatal behaviour determines gaseous flux, guard cells are a target for manipulation to improve plant water use. Stomata open in response to light, with a specific blue light response occurring at light levels too low to drive photosynthesis, greatly reducing water use efficiency. The aim of this research is to manipulate blue light photoreceptors (PHOTS) in GCs, to reduce stomatal sensitivity to BL. We will investigate the impact on photosynthesis and WUE under varying water availability and temperature conditions.

Cytokinin response reporter for wheat

Dr Tom Bennett and Dr Laura Dixon, University of Leeds

We aim to create a wheat version of the widely used cytokinin-responsive TCSn:GFP reporter gene as a tool to understand cytokinin response and distribution during wheat development. This will be a valuable tool for the emerging wheat development community and will help to understand how fine-tuning cytokinin responses in elite wheat varieties could lead to changes in shoot architecture or increased leaf-longevity (e.g. by decreased leaf senescence).

Manipulating electron transport in guard cell (GC) to determine the role of GC chloroplasts in wheat

Prof. Tracy Lawson, University of Essex

As stomatal behaviour determines uptake of CO₂ for photosynthesis and water loss via transpiration, close coupling of stomatal conductance with mesophyll demands for CO₂ is important for photosynthesis and

water use. Guard cells contain chloroplasts that are similar to mesophyll cells (Lawson, 2009), however, their role is unknown. The aim of this research is to manipulate electron transport exclusively in the guard cells to assess their importance in osmoregulation, photosynthesis and the relationship between photosynthesis and stomatal conductance.

Investigating NUE via NR1 regulation

Dr Laura Dixon and Dr Tom Bennett, University of Leeds, Dr Stephanie Swarbreck, NIAB

Increasing the sustainability of agriculture is an important environmental and economic goal. One method for facilitating this is to increase the nitrogen use efficiency (NUE) of our major cereal crops. To achieve this, we need to understand how the genes involved in NUE are regulated and how increased NUE would affect other important aspects of plant development and nutrient assimilation. We will investigate this through use of tagged NITRATE REDUCTASE 1 in spring wheat.

Understanding penetration defence failure towards rice blast

Dr Michael Deeks, University of Exeter

Many crop diseases begin with the physical penetration of a plant cell. This one point of failure in plant defence is the gateway to successful microbial infection. This is true for rice blast, one of the most globally damaging plant diseases. This project will provide a powerful new tool derived from research in *Arabidopsis thaliana* to probe why penetration defence fails against this deadly disease. This will have impact for both applied and fundamental plant science.

Wheat transformation to improve thermotolerance and mitigate the negative impact of high temperature events on yield and quality

Prof. Cristobal Uauy, John Innes Centre, Javier Canales and Francisca Castillo, Universidad Austral de Chile

Using RNA-Seq we identified a rotamase gene which is strongly induced in wheat grains under heat-stress in field experiments. Functional studies in *Arabidopsis thaliana* have shown that rotamases are essential for thermotolerance. However, the biological functions of these genes in wheat remains unknown. We propose overexpressing a rotamase gene in wheat to test the hypothesis that high levels of expression will confer thermotolerance to heat shock events, thus mitigating the negative impact on grain yield and quality.

Characterisation of gene controlling rice seed vigour

Dr Peter Eastmond, Rothamsted Research

Seed vigour is important for good crop establishment when cultivating rice by direct seeding. Many Asian farmers are transitioning from puddled transplanted rice to forms of direct seeding because labour and water availability are in decline and their associated costs are rising. We have performed a genome wide association study on ~700 rice accessions and identified a gene that we believe controls seed vigour. The aim is to perform a molecular complementation to show causality and aid in gene characterisation.

Biofortification of wheat grains with betalains for improved human nutrition and fungal pathogen resistance

Dr Samuel Brockington and Dr Hester Sheehan, University of Cambridge, Prof. Alison Bentley, NIAB
Betalains are plant pigments with high antioxidant activity and other health-promoting effects. There are few sources of these pigments in food crops, due to their limited distribution in the plant kingdom. We propose to produce betalains in the endosperm of wheat in order to study the effect of these pigments in two ways: 1) to test if these compounds improve key markers of human health and nutrition, and: 2) to evaluate whether these pigments confer protection against fungi in wheat.

Investigating the role of the circadian clock in rice blast disease using an arrhythmic RNAi rice line

Dr George R. Littlejohn & Ciaran Griffin, University of Plymouth

Rice blast disease, caused by the fungus *Magnaporthe oryzae*, destroys enough rice to feed 60 million people annually. We have seen that the time of day at which infection occurs has a dramatic effect in determining the disease severity and that *M. oryzae* mutants in circadian clock function are impaired causing disease in rice plants. This project will selectively silence the rice OsPCL1 gene, thereby disrupting the host circadian clock and assess the resultant lines for *M. oryzae* susceptibility.

Investigating the role of the circadian clock in rice blast disease using a luciferase reporter line

Dr George R. Littlejohn & Ciaran Griffin, University of Plymouth

Rice blast is a devastating fungal disease, destroying enough rice to feed ~60 million people annually. Many pathosystems show time of day-dependent infection outcomes, where plants are better equipped to defend themselves, or pathogens are more virulent at

certain times of day. Previous entrainment to environmental conditions may alter the rice immune response. Development of an pOsPCL1 luciferase reporter line in rice will allow us to track the rice clock in real time prior to and during rice blast infection.

Candidate genes for nematode resistance in rice.

Prof. Adam Price, University of Aberdeen

Genetic mapping of very rare resistance of two rice cultivars to the root-knot nematode *Meloidogyne graminicola* has identified a small region of chromosome 11. Bioinformatic investigation of available sequence has identified two candidate genes that fulfil two criteria- they are like known nematode resistance gene and the resistant cultivars have the same alleles that are exceptionally rare in rice (frequency < 0.002). We want to use CRISPR to knock these genes out in at least one of the resistant cultivars.

Deploying the Arabidopsis immune receptor MIK2 in wheat to combat Fusarium infection and associated mycotoxins.

Dr Peter van Esse, The Sainsbury Laboratory, Norwich

Immune receptors localised on the plant cell-surface enable plants to perceive pathogens and mount a defence response. The Brassicaceae-specific cell-surface receptor MIK2 contributes to Fusarium resistance as it enables recognition of Fusarium elicitors. We hypothesise that transfer of MIK2 from Arabidopsis into wheat may confer enhanced Fusarium resistance by expanding the ability of wheat to detect this pathogen. Our goal is to sustainably reduce cereal losses to Fusarium infection and mycotoxin levels within the food system.

Increasing wheat grain size through pectin modification

Prof. Simon McQueen-Mason, University of York

We previously showed that wheat grain yield can be increased by 11% by overexpressing expansins during grain development. This approach requires the use of GMOs. Expansins increase cell wall extensibility, whereas pectins decrease cell wall extensibility and other work has shown that decreasing pectin can increase growth. We propose to increase grain size by decreasing pectin content during grain development by knocking out pectin biosynthetic genes. If successful, this will open the way for non-GM approaches to increase grain yield.

Generating a platform for assembling an algal CO₂-concentrating mechanism in rice

Dr Alistair McCormick, University of Edinburgh

Ribulose 1,5-bisphosphate carboxylase/oxygenase

(Rubisco) is the driving force behind CO₂ assimilation in plants and a key engineering target for improving C3 crop yields. One promising strategy to increase

Rubisco efficiency is to introduce an algal CO₂-concentrating mechanism (CCM). This project aims to transfer current progress from Arabidopsis into rice by engineering a key requirement for assembly of the algal CCM – expressing the small subunit of Rubisco from the green alga *Chlamydomonas reinhardtii* and removing the five native small subunits.

Suppression of wheat defence by fungal leucine-rich repeat proteins

Dr Graeme Kettles, University of Birmingham

Plant immune systems are powered by cell-surface and intracellular immune sensors that frequently contain leucine-rich repeat (LRR) domains. These domains facilitate recognition of pathogen-derived molecules and help trigger defence activation. Numerous fungal pathogens are predicted to secrete LRR domain-containing proteins into plant tissues during infection. This project will test whether expression of fungal LRR-domain containing proteins in wheat suppresses ability of wheat plants to mount defence responses.

Does overexpression of SbAquaporin like gene enhance salt and drought tolerance in wheat?

Dr Dylan Phillips & Prof. Huw Jones, IBERS, Aberystwyth University

To mitigate the threat posed by salinity, research into the mechanisms that enable salt-tolerant plants to survive in this challenging environment is required. The study of halophytes that grow in highly saline soil and drought conditions could be a means to resolve this issue. A gene predicted to endow the halophytic character has been isolated and characterised from *Salicornia brachiata*, and will be overexpressed in wheat and salt-tolerance assayed.

Shape shifting stomata: the role of geometry in plant cell function

Prof. Andrew Fleming & Prof. Julie Gray, University of Sheffield

The essential role of grass stomata in controlling plant photosynthetic gas exchange and water use in cereal crops makes understanding their function of vital importance. Using cellular geometry data extracted via confocal microscopy and image segmentation, we aim to generate a 3-dimensional mechanical model of grass stomata with which we hope to address how a 4-celled system (as found in grasses) is able to confer superior performance, with associated improvements

in water-use efficiency, over the 2-celled system found in eudicots.

Enhancing hypoxic germination in wheat

Dr. Francesco Licausi

Flooding conditions pose a grave threat to food security, as they cause major yield losses worldwide. Most crops are susceptible to this stress, especially at the germination stage, due to restricted oxygen availability underwater. Indeed, most cereals, including wheat, are unable to germinate under strict hypoxia. By merging the advances of plant genetics, physiology and synthetic biology, we devised a strategy to implement this trait in wheat, thus enabling this crop to cope with the impending climate change.

Common mechanisms in pathogenesis and symbiosis

Prof. Uta Paszkowski, University of Cambridge

The rice blast fungus *Mangarthe oryzae* causes the most devastating fungal disease of cultivated rice. *Rhizophagus irregularis* on the other hand belongs to the wide-spread beneficial arbuscular mycorrhizal fungi that readily colonise most land plants, including all cereal crops. Both fungi infect rice roots and invaginate the plant plasma membrane for hyphal intracellular proliferation. On the side of the host, common mechanisms must exist to accommodate hyphae of either filamentous fungus inside root cells. Comparing the transcriptome of rice roots in response to colonisation by either fungus revealed a number of genes as being co-upregulated. Three of these were selected on the grounds of having no detectable expression in the absence of fungal root colonisation but show high levels of transcripts in colonised roots. These genes are predicted to encode a DUF538 protein, a Lectin Receptor-like kinase (LecRLK) and a member of the Exo70 family. To address the functional relevance of these genes, a CRISPR/Cas9 approach has in the past been initiated for the DUF538 and the resultant transformants are currently characterised for the occurrence of edit-events. Here we wish to extend our functional analysis and request rice transformation to knockout the rice LecRLK gene via CRISPR-editing. The second request for rice transformation focuses on the EXO70 gene to complete the functional assessment of the top candidate genes.

Manipulating gene expression of ASY1 to remodel meiotic recombination and the pattern of genetic diversity in wheat

Dr. Christophe Lambing, Prof. Malcolm Hawkesford, Rothamsted Research

ASY1 is a component of the chromosome axis and

mediates reciprocal exchanges of genetic information between homologous chromosomes which create novel genetic diversity during meiosis. Arabidopsis

ASY1 is enriched towards the centromeres and promotes high recombination in adjacent regions. Reduction of ASY1 gene expression causes a redistribution of recombination towards the telomeres. In wheat, recombination is exclusively located in chromosome ends where ASY1 is enriched. We propose to over-express ASY1 to remodel its distribution and manipulate the recombination landscape.

Generating knockout line of the master regulator ATR kinase to increase meiotic recombination in wheat

Dr. Christophe Lambing, Prof. Malcolm Hawkesford, Rothamsted Research

ATR kinase is a master regulator regulating the response of DNA repair. During meiosis, programmed-DNA double strand breaks are repaired using the homologous chromosome as template resulting in DNA crossovers and formation of new allele combinations. *atr* Arabidopsis showed hyper inter-homologue recombination activity associated with an elevation of crossovers throughout the genome. In contrast, wheat crossovers are restricted to the distal chromosomal regions. We propose to knockout ATR in wheat to increase the crossover events and create novel genetic diversity.

Manipulating leaf venation architecture to address plant performance

Prof. Jane Langdale and Dr Daniela Vlad, University of Oxford

This project aims to manipulate leaf venation architecture in wheat with a view to modifying water uptake properties and/or photosynthetic capacity.

Overexpression of an Arabidopsis IQD gene conferring drought resistance in a monocot

Dr Michaela Matthes and Dr Smita Kurup Rothamsted Research

In view of the predicted consequences of climate change the generation of crops with enhanced resilience to drought is becoming paramount. We have identified a novel IQD gene from Arabidopsis which, when constitutively overexpressed, confers a 'succulent' phenotype to the transgenics with a much improved ability to withstand drought. The same effect is observed upon transfer of this IQD in other dicotyledonous species (*Camelina sativa*, poplar, tomato) but whether monocots are similarly altered

remains to be determined and will be addressed in this proposed work.

Expression of a mutant form of an enzyme that enhances lipid content in wheat

Dr Peter Eastmond

Lipids account for ~3% of dry weight in grasses. There is an incentive to breed grasses with lipid content up to ~6% to improve ruminant livestock productivity and suppress methane emissions. Using wheat as a model grass, we have identified a gain-of-function mutant in a lipid biosynthetic enzyme that's associated with an increase in leaf lipid content. The aim is to test if the mutation is causal by transforming wheat with a copy of the mutant allele.

A role for small interfering RNAs in modulating arbuscular mycorrhizal symbiosis in rice

Dr Ronelle Roth, Department of Plant Sciences, University of Oxford

Most land plants form beneficial partnerships with soil-dwelling arbuscular mycorrhizal (AM) fungi. AM symbiosis enhances crop productivity offering an environmentally sustainable alternative to chemical fertilizers. We recently discovered that a distinct class of small interfering RNAs (siRNAs), produced by Dicer-like 2, is needed to maintain AM symbiosis in tomato. This provided a first indication that siRNAs modulate the interaction. In this proposal we will examine if the role of DCL2-dependent siRNAs is conserved in the monocot cereal rice.

Further details and project summaries will be added to this list as additional applications are accepted in the CRWRT resource.