

# OpenPlant Fund Application Form

## Title of Project

Harvesting the genetic value of interspecific wheat introgressions

## Primary contact for the team

Include name, department, organisation and email address

Tobias Barber

National Institute of Agricultural Botany (NIAB) and Cambridge University Department of Plant Sciences

[toby.barber@niab.com](mailto:toby.barber@niab.com)

## Team

Please include the names and email addresses of all team members, include their department/organisation and briefly (1-2 sentences) what they will contribute to the project.

Tobias Barber

Tobias Barber (TB; NIAB and Cambridge University) is a BBSRC-funded Doctoral Training Program PhD student using genomic methods to accelerate genetic gain in UK winter wheat. In particular he is exploring the significance of interspecific introgressions (resulting from wide crosses with wild relatives) in wheat with a view to their refinement and targeted use in breeding programs [toby.barber@niab.com](mailto:toby.barber@niab.com)

Dr Alison Bentley (AB) is the Director of Genetics and Breeding at NIAB and has a research track record in wheat pre-breeding and trait dissection. This includes work encompassing the analysis of multi-site wheat data for the application of both association mapping and genomic selection and the manipulation of flowering-time and exploitation of synthetic hexaploid wheat. She has also been involved in the development of multi-parent advanced generation inter cross (MAGIC) populations for fine mapping in winter wheat and contributes to their ongoing exploitation. [alison.bentley@niab.com](mailto:alison.bentley@niab.com)

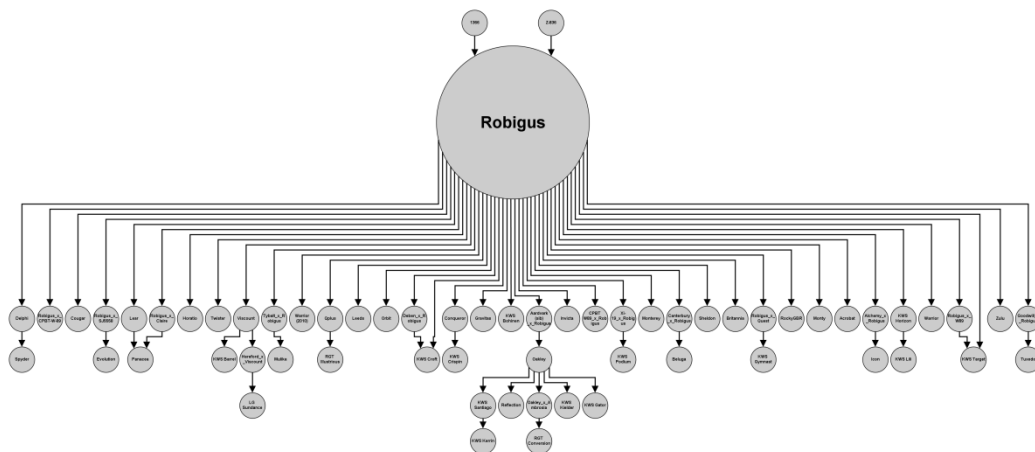
Dr Keith Gardner (KG) is a statistical geneticist at NIAB and lead author of the MAGIC mapping publication which identified the locations of interspecific introgression blocks in the MAGIC population (Gardner, K.A., Wittern, L.M. and Mackay, I.J. 2015. Plant Biotechnology Journal DOI: 10.1111/pbi.12504). He has extensive experience of analysing large wheat datasets including MAGIC populations, association mapping panels and genomic selection datasets. [keith.gardner@niab.com](mailto:keith.gardner@niab.com)

Earlham Institute (EI) will perform Illumina sequencing on two flow sorted wheat chromosomes within the proposed project. The original contact for this is Dr Dan Swan who will soon be leaving EI. We have been liaising with Dr Chris Wright regarding the project specifications and will continue to work with him. [christopher.wright@earlham.ac.uk](mailto:christopher.wright@earlham.ac.uk)

Dr Jaroslav Dolezel (JD) from the Centre of Plant Structural and Functional Genomics, Czech Republic, has been pivotal in the sequencing of the wheat genome. The flow sorting of chromosome 4A will be contributed in-kind.

Briefly summarise your proposal

Incorporating genes from wild relatives has played a major role in the breeding of modern wheat varieties. In particular, many important disease resistance and yield enhancing genes have been successfully transferred from wild grass species into the cultivated wheat gene pool. Historically this process has been rather haphazard, achieved via the process of wide crossing and phenotypic selection in breeding populations. As a result, deleterious traits, such as disease susceptibilities are often also brought in together with the positive genes. The advantages (and disadvantages) of unintended transfers often only become apparent late in the breeding process making it difficult to precisely target them. Modern genomic technology offers the potential to refine this process by locating and disentangling the specific genes carried over in these introgressions in order that they can be used to accelerate breeding in a targeted manner. In particular we are interested in the genomic composition of variety Robigus, which carries introgressions from the wild species *Triticum dicoccoides*. Robigus has played a major role in the pedigrees of modern wheat varieties, bringing in a suite of four disease resistances, as well as yield enhancements, although also carrying some deleterious genes. Robigus is a major component of UK wheat varieties (see below), and its progeny include the variety Oakley, which is the focus of TB's PhD, another significant variety.



NIAB have developed a highly recombined, diverse Multi-parent Advanced Generation InterCross (MAGIC) population (Mackay et al., 2014). From the NIAB 8-parent MAGIC population, 6 major introgressions have been found in the modern UK wheat gene pool and we have generated pairs of near-isogenic lines (NILs) which differ only at these introgressions. Two of these introgressions have come from the variety Robigus. Our preliminary work shows favourable phenotypes associated with the two Robigus introgressions and partners in France (INRA; Arvalis) have also identified a previously unknown “Robigus-effect” for nitrogen deficiency tolerance, which is hypothesised to be located on one of these introgressions.

The aim of this project is to flow sort (via an in-kind collaboration with Dr Jaroslav Dolezel at the Centre of Plant Structural and Functional Genomics, Czech Republic) and then sequence (using the requested OpenPlant funds) wheat chromosome 4A for a NIL pair extracted from the NIAB MAGIC population and differing by presence of a Robigus-derived introgression. A thorough bioinformatic analysis will then enable characterization of the

genes present in the introgressions, and establish the most likely candidates responsible for the phenotypic effects observed to be caused by the introgressions. As well as establishing the mechanistic basis of many important traits, this analysis will enable us to develop the tools to track and maintain the beneficial genes in the modern gene pool, while eliminating the detrimental genes. We anticipate it may also enable us to identify novel targets for CRISPR/Cas9 gene-editing.

## **Proposal**

Describe what you are planning to do with the funding, including aims, methods, outcomes and who will be involved

### **Aims**

The introgression of genetic material from wild relatives of wheat into breeding germplasm has played a critical role in the modern history of wheat breeding. Altogether, DNA from at least 52 other species has been introgressed into the wheat gene pool. While successful, the process of introgression has been haphazard; in particular, introgressed blocks of DNA often do not recombine well with the native wheat germplasm; these introgressed blocks can be long and frequently contain genetically linked alleles associated with deleterious traits in addition to the intended target locus. Here we aim to use flow sorting to isolate a specific chromosome of interest (in order to reduce the complexity of wheat's hexaploid genome) and next-generation sequencing in order to ultimately locate and dissect favourable (and unfavourable) interspecific regions and to facilitate their manipulation for breeding gain.

### **Methods**

#### **1. Existing NIAB resources**

NIAB have developed an 8-parent wheat MAGIC population, which captures c. 80% of the genetic variation found in UK wheat varieties. When constructing a genetic map from this population, a high degree of segregation distortion (SD) has been observed, including long (>20cM) linkage blocks of markers. Six of the longest and most distorted blocks are co-linear with non-centromeric high marker density regions of the genome, the signature of an inter-specific introgression fragment. The two most distorted blocks both involve the variety Robigus – with the block on chromosome 4AL, with SD against Robigus alleles, being of interest to this project. The Robigus blocks have been validated using the Bristol University 820K SNP array database. Robigus is a key component of the pedigree of a large percentage of contemporary wheat varieties (as above) and favourable traits are thought to have been derived from introgressions from *Triticum dicoccoides* in its pedigree.

#### **2. Emerging NIAB resources**

NIAB has generated NIL pairs for all 6 of the MAGIC introgressions from heterogeneous inbred F5 MAGIC families (HIFs), allowing for direct testing for phenotypic effects of the introgressions for any trait of interest. Furthermore, we have additional populations segregating for both Robigus introgressions. Preliminary data has been gathered on the potential phenotypic effects of the Robigus introgressions. So far, a negative yield effect associated with the 4A introgression (from 3 sources) has been found. There is strong evidence for brown rust resistance associated with the 4A introgression (from 2 sources), and some evidence for yellow rust susceptibility associated with the same introgression. Additional preliminary data suggest a locus affecting seed size and shape (and possibly weight) is found in the 4A introgression. This data has allowed us to prioritise the 4A Robigus introgression for further fine-scale investigation.

#### **3. OpenPlant project**

In this project we will sequence the entirety of chromosome 4A for the NIL pair, which will

generate the full sequence of the most significant Robigus-derived introgression. A thorough bioinformatic analysis will be conducted to annotate and characterize all the genes present in the introgression, and establish the most likely candidates responsible for the phenotypic effects observed from in the introgression NIL pairs.

### **Outcomes**

This project will allow us to characterize, to a candidate gene level, the genes responsible for several important traits in wheat. It represents a novel approach of detecting and dissecting interspecific introgressions in wheat using a combination of modern genomic technologies with germplasm resources derived from multi-parental populations, with the aim of maximizing the potential benefits of interspecific introgressions in the wheat gene pool to the benefit of breeders and farmers. Overall this project will maximise the benefits of these interspecific introgressions resulting in improved combinations of disease resistance, nitrogen use efficiency and yield in UK wheat varieties.

### **Who will be involved**

The proposed project will be led by NIAB/Cambridge PhD student TB with support from AB and KG. TB is currently growing and field phenotyping the 4A NILs and will be responsible for extracting high-quality DNA for flow sorting. The flow sorting of chromosome 4A will be contributed in-kind by Dr Jaroslav Dolezel at the Centre of Plant Structural and Functional Genomics, (Czech Republic) who has been pivotal in the sequencing of the wheat genome. Library preparation and Illumina sequencing will be done by collaborators EI and all of the transferred data will be analysed at NIAB by TB with support from KG and his group.

### **Benefits and outcomes**

Describe how your project fits the remit of OpenPlant and the judging criteria, including details of any new interdisciplinary interactions between Cambridge and Norwich.

TB is a NIAB and University of Cambridge PhD student. NIAB is a partner institute of the University of Cambridge. NIAB have several collaborative genomics projects ongoing with Earlham Institute who will conduct the sequencing.

### **DNA sequence characterization of interspecific introgression in wheat, including annotation and characterization of genes contained therein.**

This will include understanding whether the introgressions contain novel alleles at genes found in bread wheat or whether they are genes entirely absent in bread wheat. In addition, we will confirm the specific origin of the Robigus introgressions by comparison to the recently completed *Triticum dicoccoides* genome.

**Determination of which genes in the introgressions underlie the trait effects** from field phenotypic data and the molecular mechanisms involved and whether novel genes or just novel alleles are involved.

**Development of specific markers for these genes** to allow their rapid and targeted use in breeding in support of UK wheat improvement

### **Sponsor for the research and cost centre**

Dr Alison Bentley  
Director of Genetics and Breeding, NIAB  
Alison.bentley@niab.com

*I confirm that I have the full support of the sponsor listed above and that they can be added to the OpenPlant Fund mailing list to receive project updates (to which they can unsubscribe at any time).*

### **Budget**

Provide costings for your proposal (up to £4000) and indicate if you have access to any additional funding to meet your aims.

We have obtained a formal quote from EI (ENQ-2717) for the construction of two PCR free libraries and sequencing on 1 lane of 250 bp paired end sequencing. This approach has been discussed with EI and is agreed as the best option for generating long sequencing reads and eliminating PCR duplicates.

Item	Total
PCR-free library x 2	£4,498.61
HiSeq2500_RR_lane (250PE) x 1	
Data transfer	
<b>TOTAL</b>	<b>£4,498.61</b>
VAT (If applicable)	£899.72

We request £4,000 of OpenPlant funds for this sequencing.

The additional £498.61 will be covered by NIAB.

We request an additional £1,000 to cover travel to Norwich to meet with EI staff and to attend the 2018 annual UK Cereals community meeting (Monogram) to present early results from the analysis.