

Annual Project Report October 2017 to September 2018

Project title	Developing systems to control male fertility in wheat for hybrid breeding, enhanced pollen production and increased yield.		
Project number	21130024		
Start date	1-OCT- 2016	End date	31-SEPT-2020

Project aim and objectives

Successful reproduction is critical to grain set and crop yield in cereals. Thus, optimising and enhancing fertility, alongside controlled fertilisation for breeding and hybrid development, is key to achieve high yields in a sustainable manner. Hybrid vigour increases yield, therefore, developing hybrid crops offers opportunities to increase productivity. However, this is challenging due to the need to avoid self-fertilisation, therefore, mechanisms that control fertility in a reversible manner are needed. There is also a requirement to ensure effective pollination; this relies on high levels of viable pollen for cross-pollination, which is distributed effectively and is resilient to abiotic stress.

This project aims to address these issues by providing greater understanding of pollen development in cereals by developing switchable systems for the control of wheat fertility, and also by identifying traits for enhanced pollen production and viability, particularly under environmental stress, which are critical for ensuring successful pollination in breeding programmes. We will also determine the environmental stability of our fertility control mechanisms in barley, to enable understanding of the fertility control under different temperatures and light intensities. In addition to providing tools for barley and wheat breeding, this work will help in the characterisation of the underlying mechanisms for the control of fertility. This is particularly the case due to the diploid nature of barley and the greater tools and resources available for barley genetic analysis. In addition, the work will identify the benefit of hybrids for fertility control and the stability of these systems in elite breeding materials.

Key messages emerging from the project

There is conservation of the gene networks involved in pollen development. Genes have been identified from barley and wheat that are critical for pollen development. These are being targeted by mutagenesis to help characterise their function. Gene editing (CRISPR/Cas9) is being used for two wheat fertility-associated genes to study the suitability of this technique to produce mutants with altered fertility and to help determine gene function.

Summary of results from the reporting year

1) We have obtained heterozygous lines for the three wheat male sterility genes; these have been selfed. The segregating population from this is growing now and KASP marker analysis is ongoing. From this, we will conduct temperature/photoperiod analysis to observe whether any sterility phenotypes are restored under different conditions.

2) qRT-PCR analysis is ongoing to understand homeologue expression patterns for each gene. This

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will be used to study the mutant lines and to identify potential homeologue compensation and gene redundancy.

3) KASP marker development for the target genes has been concluded and all the lines under analysis have functional KASP assays.

4) We have been analysing the impact of environmental conditions on barley over-expression Transcription Factor (TF) male sterile line.

- Transmission Electron Microscopy (TEM) of the over-expression line showed alterations in the anther wall and tapetum, which are critical in anther and pollen development. Pollen wall deposition was also impaired and irregular. Scanning Electron Microscopy (SEM) will be used to clarify these changes.
- Lipidomic analysis of developing, staged buds looked for changes in anther and pollen development. This has identified changes, which are being associated with transcriptomic changes. Further analysis on pollen samples will be carried out.
- RNAseq has been performed to carry out transcriptomic analysis of bud samples during pollen development. Bioinformatic analysis show that some interesting genes have been identified that are associated with anther dehiscence, which are downregulated in the sterile over-expression line. There are also changes associated with Programme Cell Death (PCD) pathways that are essential for stomium breakdown and pollen release. Work is in progress to determine whether there are changes associated with anther development-associated PCD, and whether this is linked to the observed male sterility in these lines.

4) Three different barley male sterility-associated transcription factors (TF) have been targeted by CRISPR/Cas9 gene editing.

- HvTF1: Up to 8 mutated T1 lines have been identified and genotyped. Lines showing sterility are being crossed with wild type lines, to rescue the material and test female fertility.
- HvTF2: Only three plants were found with high-levels of male sterility, however, full genotyping of these lines is proving difficult. The T1 lines are growing and will be crossed with wild type to eliminate the transgene. A new round of transformation with new targets to obtain more lines is ongoing.
- HvTF3: This mutant was developed at JIC. Only one line showed gene editing. The T1 is now growing and genotyping has confirmed the gene editing. Phenotype is being conducted and crosses with wild type have been carried out. A new set of transformations with new targets to obtain more gene edited lines is ongoing.
- The technique is being used by a number of students (trained by JFG) for characterisation of additional multiple genes.

5) Cadenza wheat staging systems have been completed and manuscript is under preparation. A

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further manuscript is in preparation relating to TF during pollen development.

6) HvTF1 over-expression introgression into commercial lines has been completed; these will be tested for their fertility and ability to outcross.

7) Introgression of TILLING mutations into elite wheat lines is ongoing; KASP marker analysis is being used to identify mutants and lines with multiple mutations.

Key issues to be addressed in the next year

- CRISPR-effective genotyping.
- Characterise mutant gene expression, and homeologue compensation.
- Characterise wheat triple homozygous lines for male fertility and the reasons for it.

Lead partner	Prof Zoe A Wilson; University of Nottingham
Scientific partners	-
Industry partners	KWS, LIMAGRAIN, RAGT, SECOBRA
Government sponsor	BBSRC

Has your project featured in any of the following in the last year?

Events	Press articles
Breeding Partners Dissemination Meetings 24 th Nov 2017; 12 th July 2018	N/A
Conference presentations, papers or posters	Scientific papers
Presentation: José Fernández-Gómez, Behzad Talle and Zoe A Wilson (2018) Environmentally sensitive control of male fertility in barley. iBMW, Dundee. Poster: Characterisation of pollen development genes in barley using CRISPR. (2018) Johanna Astrand, Jose Fernandez Gomez, Zoe Wilson. iBMW, Dundee.	Two manuscripts under preparation
Other	