

Annual Project Report October 2018 to September 2019

Project title	Developing systems to control male fertility in wheat for hybrid breeding, enhanced pollen production and increased yield		
Project number	21130024		
Start date	01/10/2016	End date	30/09/2020

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 will address these issues by providing greater understanding of pollen development in cereals by developing switchable systems for the control of wheat fertility, but 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 fertility control mechanisms in barley and wheat, to enable understanding of the fertility control under different temperatures and light intensities. In addition to providing tools for barley breeding, this work will help in characterisation of the underlying mechanisms for the control of fertility. This is important due to the diploid nature of barley and the greater tools and resources available for barley genetic analysis. Therefore, by investigating the mechanisms behind these traits and by generating tools for breeding and selection, effective breeding to increase crop productivity and resilience will be realised. The work will also 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 and to generate male sterile lines that could be used for hybrid breeding approaches. In addition, gene editing is being tested for one representative gene, to study the suitability of this technique to produce same phenotype as those generated by conventional mutagenesis.

The results described in this summary report are interim and relate to one year. In all cases, the reports refer to projects that extend over a number of years.

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Summary of results from the reporting year

1. We have obtained homozygous (Hm) lines for all the homoeologs for all three wheat (Ta) pollen transcription factors (TF) under investigation. The three homozygous lines have been grown and backcrosses are ongoing. Two of these have shown clear male sterile phenotypes, however, one of these seems to have some restoration of male fertility when grown at different temperatures. The other showed an abnormal growth phenotype that is being investigated. Analysis of growth and fertility under different temperature and photoperiod regimes are ongoing for the three lines.

2. qRT-PCR was developed to determine homoeologs expression pattern for each gene. This will be used to study the mutant lines and to compare potential homoeolog expression compensation. Plant samples will be collected after backcross 2. In addition, once triple homozygous lines have been phenotyped, we will select different combinations of homologue mutations to observe the relative importance of some homologues over others. In addition, we will grow those mutant combinations that are sterile at different conditions to observe whether fertility can be restored.

3. We have been analysing the impact of environmental conditions on our barley over-expression male sterile line:

- i) TEM showed increased anther wall thickness and a delay/lack of tapetum layer degeneration within the anther, with irregularly arranged Ubisch bodies. This will be clarified by SEM analysis.
- ii) RNAseq analysis has identified some interesting genes essential for anther dehiscence that appears to be downregulated in the sterile overexpression line. Programme Cell Death (PCD) analysis was appeared normal, although slightly advanced in overexpression tissues. PCD in the anther septum and stomium seems normal.
- iii) Lipidomic analysis of anthers and pollen has been carried out and results are being associated to the RNAseq and SEM data.
- iv) Ultrathin sectioning showed that tapetum has differential temperature dependant degeneration.

4. Three different barley Transcription Factors have been mutagenised by CRISPR gene editing; these have been genotyped and phenotyping is ongoing. Crosses with wt were conducted to segregate out the CAS9 construct. Four T1 segregating lines showed no CAS9 and Hm/Het mutation mix. Hm lines are growing at different temperature to observe any changes in male fertility/sterility.

5. HvTF1: From the original three plants showing gene editing in T0, only one showed Het/Hm genotype at T1. Extra targets have been inoculated and only target 6 showed three plants with gene

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editions in T0. T1 will be grown promptly. T1 homozygous lines are growing and showing complete male sterility. This line is being crossed with wt to maintain it and to segregate the Cas9 out.

6. HvTF3: This line was developed at JIC. Only one line showed gene editing. T1 showed a Hm genotype and its phenotype was similar to previous obtained for the same gene in barley using other gene expression reduction methods. Plants showed multiple tillers and dwarfism. Crossing the plants was impossible due to the size and pollination occurring within the last sheath; lines were not completely sterile.

7. A wheat Cadenza floral development staging systems was completed, and a manuscript describing this is under review. In addition, two additional manuscripts on HvDYT1 and HvMS1 overexpression are in progress.

8. The HvTF1 over-expression construct has been introgressed into elite barley lines; cross-pollination experiments produced seed; this was assisted due to the floral gapping observed in these sterile lines.

Key issues to be addressed in the next year

- Characterise wheat triple homozygous lines in elite backgrounds
- Analyse potential homologue expression compensation in mutant backgrounds.
- Microscopy and functional analysis of mutant lines.

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?

<i>Events</i>	<i>Press articles</i>
<i>Conference presentations, papers or posters</i>	<i>Scientific papers</i>
Presentation at Net4FS EU Mobility programme Dissemination Event, SJTU, China July 2019	One currently under review

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