

Project title	A 'breeder's tool kit' to improve Hagberg Falling Number for the economic and environmental sustainability of UK wheat		
Project number	RD-2009-3659	Final Project Report	PR579
Start date	Nov 1, 2010	End date	Nov 1, 2014
AHDB Cereals &	£120,000	Total cost	£1,340,020
Oilseeds funding			

What was the challenge/demand for the work?

The aim of this programme was to generate knowledge and tools to help develop new wheat varieties with increased and more stable Hagberg Falling Number (HFN) under variable weather conditions. This project built upon the results from a previous AHDB-Defra-LINK investment (HGCA project 480) and sought to translate the knowledge generated from the initial discovery phase into a practical 'breeder's tool kit'.

How did the project address this?

To address this we further characterized the largest and most stable regions of the wheat genome (known as Quantitative Trait Loci or QTL) which were proposed to affect HFN under UK environments. This included understanding the mode of action of each QTL, understanding any negative side-effects on yield and agronomic traits associated with the QTL, generating genomic information for each QTL, and defining them to sufficiently small regions in the wheat genome to allow marker-assisted selection by breeders. Ultimately, we aimed to produce the knowledge and the tools to allow breeders to purposely combine and deploy the genes responsible for different modes of resistance to HFN using the latest genomics and molecular marker technology.

What outputs has the project delivered?

- We identified a major gene affecting pre-harvest sprouting in UK wheat (Figure 1a). We translated this knowledge into a breeders' toolkit by developing a high-throughput perfect SNP marker which allows breeders to tag the mutation which confers resistance to pre-harvest sprouting. This information is now being implemented by UK breeders to deliver varieties with enhanced sprouting resistance to UK industry.
- We have further prioritised two additional genes which confer improvements in HFN values in UK wheat. Both genes were confirmed and showed stable effects across multiple trials across years and locations. We show that these genes do not affect yield in a series of trials which is a critical consideration when evaluating their deployment into elite varieties. Across experiments these genes provide an increase in HFN of 25 and 32 s, respectively (Fig 1b). Importantly, these genes have distinct modes of action. This suggests that combining both genes could lead to average increases in HFN of over 50 s in UK wheat. Similarly, combining both genes provides complementary resistance mechanism that could be relevant depending on weather events for the

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particular year and location where the variety of grown. Both genes have been mapped to relatively small genetic intervals and breeder friendly markers have been developed and transferred to industrial partners. This will enable rapid targeted deployment into UK elite varieties.

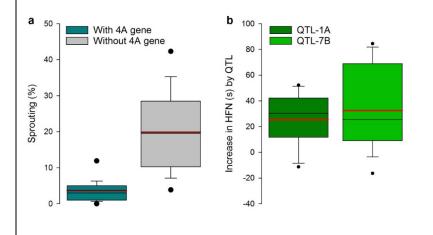


Figure 1: Summary of gene effects. (a) The 4A gene (teal) leads to decreases in sprouting in late harvested field samples. (b) The 1A and 7B QTL lead to increases in HFN across multiple sites and years (n=11 and 18). The boundaries of the box indicate the 25th and 75th percentiles; the black line marks the median (50th); the red line marks the mean (average).

- As an example of how this information can be taken forward, we screened and categorised UK germplasm based on the causal marker for the 4A gene (called *TaMKK3*). This marker is an example of the high-throughput assays that breeders can now use to classify lines for sprouting resistance based solely on DNA sequence and reducing the amount of destructive phenotyping of new germplasm. We developed detailed pedigree trees of UK varieties and categorised them based on this gene.
- We also identified different versions of the wider chromosome region (called haplotypes) and categorised UK varieties based on the gene (Figure 2a, centre graph) and these wider chromosome regions (side graphs). This shows that there are three major haplotypes in UK varieties (Haplotypes 1-3-5). Examination of RL varieties showed that 85% of recent Group 1 and 2 varieties carry the resistant version of this gene (n=13), whereas only 35% of Group 3 and 4 varieties have the resistant allele (n=28; Figure 2b). This could be due to the fact that key parents of group 3 and 4 varieties (such as Claire, Robigus, and derivatives such as Nijinsky and Oakley) carry the susceptible version of this gene. This analysis now allows breeders to understand how specific genes and chromosome regions affecting HFN are being deployed in UK varieties (Figure 2c).
- The 4A *TaMKK3* gene confers protection to late induction of sprouting as the gene affects the rate in which seeds lose dormancy. Hence the protective allele provides a delay in the loss of dormancy at the end of grain development. This means that seeds will not germinate in cases were late rains disrupt or delay the harvest of wheat crops. However, this protection is sufficiently short lived given that there is no effect in seed germination for commercial sowings a few months or even weeks later.

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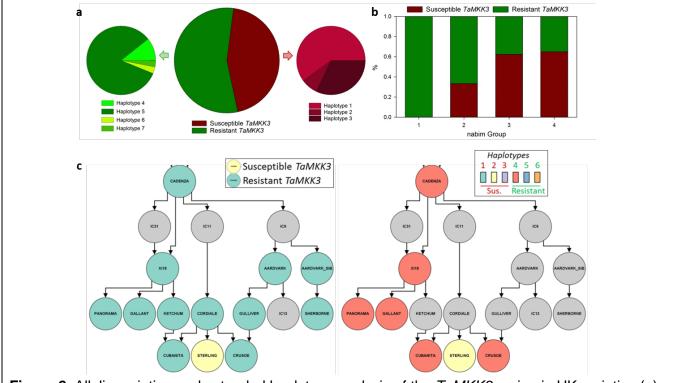


Figure 2: Allelic variation and extended haplotype analysis of the *TaMKK3* region in UK varieties (**a**) Centre chart shows the *TaMKK3* allele frequency and side charts show the breakdown of Haplotype groups (green: resistant; red: susceptible). (**b**) Frequency of two allelic variants at *TaMKK3* for 41 RL varieties classified according to their nabim Group. (**c**) Pedigree of Cadenza derived varieties for *TaMKK3* (left) and the chromosome haplotype (right). Group 1 and 2 varieties (Xi19, Panorama, Gallant, Cubanita, Crusoe) carry the resistance gene (teal circles) and have been selected for Haplotype 4 (red terra cotta circles).

- Our results suggest that combining these three genes (1A, 4A, 7B) could provide an opportunity for robust protection against low HFN values for UK wheat. Given the way these genes work, they should provide complementary and additive resistance. Importantly, they could constitute the equivalent to an insurance against different weather events that could trigger low HFN values.
- We have also developed new tools and strategies to improve the speed of transfer of technologies arising in the academic sector into functional kits and assays that can be routinely implemented in breeders' laboratories. This has accelerated the rate in which new information gained from the wheat genome is being transferred to breeders and into new varieties with a direct benefit to UK growers.
- This project, alongside advances by others in the field, has now made marker-assisted selection for high HFN a reality in UK wheat breeding programmes.

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Who will benefit from this project and why?

The financial benefits of this project will arise by farmer adoption of wheat varieties with improved HFN stability. These benefits will be realised as breeders are able to translate the information and tools generated in this project into commercial wheat varieties for UK farmers. The direct costs of failing HFN thresholds of 250 s for group 1 wheat is £20.81 per T which is the average premium for bread making wheat over feed wheat between 2010 and 2016. The average risk of nabim group 1 failing to meet the 250 s HFN threshold is between 1 in 4 and 1 in 5 (20-25%) but also there is extreme volatility ranging from 2% of in 2013 to 62% failing in 2012. This has important financial consequences for the sector and on-farm profitability. There are the direct costs to farmers when selling grain (£20.81 per T), but additional costs arise from the increased nitrogen fertilizations required to meet protein specifications in bread making wheat and the loss of income given the reduced yields typically associated with group 1 wheat varieties. These extra costs are offset by the £20 per T premium for bread-making wheat, but this incentive is lost if the harvested crop falls below the HFN threshold. We have identified and characterised complementary sources for HFN resistance and developed the breeders' tool kit required for their deployment. Together, this information and tools will enable the breeding of new wheat varieties with increased and more stable HFN and reduced environmental impact, thereby reducing the costs associated with low HFN events.

If the challenge has not been specifically met, state why and how this could be overcome

Three of the QTL were difficult to define either because they did not confer consistent levels of resistance or because they mapped to positions on the chromosome that made further work extremely complicated. These limitations cannot be overcome as the biological effect is too subtle to define, and hence not of sufficient value to growers, whereas the complex chromosome position of one gene would require additional investment that would not be value for money. The three validated and well defined genes, however, provide breeders with a strong and robust toolkit to breed for improved sprouting/HFN resistance using marker assisted selection as originally proposed.

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