HGCA

from theory to field

Breeding route to better bread?

New research into plant genomics has led to significant advances in understanding how to produce a quality wheat. *CPM* explores what this means for growers.

By Tom Allen-Stevens

> *"It's early days, but we are honing in on specific genes that protect UK varieties."*

It would be great to think that British growers could supply all the wheat needed for British bread. Sadly, it's some way from consistently meeting the grade, however. According to the HGCA Cereal Quality Survey, over the past five years, less than one in four Group 1 samples met the full milling spec on average, and in 2008, this dipped to just 6%.

What's more, it's not something that'll necessarily improve through advances in breeding, points out HGCA's Dhan Bhandari. "Breeders' primary aims are to maximise yield and improve disease resistance. Satisfying the criteria of end users comes lower down on the list of priorities. That's not because they're seen as less important, but because there's a wide range of factors that determine grain quality."

It's not just the quality traits – high protein, Hagberg Falling Number (HFN) and specific weight — that must be bred in. There's also consistency. "This is an important factor for millers and bakers — a variety has to perform over a range of weather conditions and seasons before it's favoured. So trying to produce such a variety is a major, major challenge."

There's also the issue of N inputs. "Milling wheat needs a lot of nitrogen to achieve the protein levels, but it's not always successful, which potentially means there's N going to waste. That's a cost to growers, but there's an environmental cost too."

New breeding technology, such as the use of genetic markers, could hold the key to some of the improvements, says Dhan Bhandari. "What's needed is help in identifying which part of the wheat genome is being influenced by environmental factors, and therefore which genes breeders need to focus on to improve wheat quality and consistency."

That's been one of the aims of HGCA-funded research into bread-making quality. Just completed is a project that has explored the sustainability of UK-grown wheat. "As yield increases, protein levels tend to drop. But there are some varieties that buck the trend. Why are they doing so and is the performance consistent? Also, what are the genetics behind it?

"This project has unravelled some of the basic genetic information and related it to environmental factors, utilising small-scale predictive tests for determining gluten quality." he reports.

A breeders' tool kit to improve HFN is currently under development in another project underway at John Innes Centre. "HFN is a major determinant of grain quality, but it varies enormously at harvest and between varieties. Some cultivars are prone to sprouting, while the HFN varies in others with no easily determinable cause, and there's no on-farm test for it.

"The aim of this project is to understand what's going on, and pinpoint the genetic locus that determines these changes. Breeders can then select for HFN with more reliability and precision."

Practical applications for research don't stop with the breeders, however, continues Dhan Bhandari. Another project, completed in 2010, explored the relationship between late urea applications and grain protein. "It's very difficult for a grower to assess the right timing of this application and whether it'll actually have the desired effect. You need a quick, reliable test to tell you.

"This project identified the technology needed, and the major breakthrough has been relating that measurement to predicted grain protein at harvest. The challenge since the project ended has been to adopt the technology on farm into a practical, useful service, and the good news is that ongoing commercial funding has taken it forward."

More fundamental research is needed to improve HFN — on average, 30% of Group 1 samples fail to reach the 250 benchmark required for the bread-making standard, and this rose to 60% in 2012. HFN is also linked to pre-harvest sprouting, so breeding advances would benefit not just the quality wheats. But Dr Cristobal Uauy of John Inness Centre believes his team may be close to finding a genetic solution, and one that's permanent.

"As with losses from disease, you can improve HFN with good agronomy, or you can improve the genetics of the variety. But if you can breed in a better HFN, that'll last forever — it won't be overcome, as can be the case with disease resistance," he points out.

Previous research had shown the variations in HFN are down to genetics in the UK germplasm. "We knew that the variation was down to regions containing hundreds of genes, but we didn't know which of these genes were responsible, and how they influenced HFN — was each gene contributing 1sec to the variety's HFN, or was one gene doing it all?"

Hagberg varies in some varieties with no easily determinable cause, and there's no on-farm test for it.

Extra applied nitrogen that doesn't result in a milling premium is N that's going to waste, points out Dhan Bhandari.

So researchers began to investigate these quantitative trait loci (QTL) — specific regions in the wheat genome responsible for building HFN. "HFN isn't like the dwarfing gene, for example, that's either there or it's not. It's more of a sliding scale," explains Cristobal Uauy.

The research team identified six QTLs and the first job was to develop germplasm that had the parental alleles across the QTL, and identical cultivars that didn't, known as near-isogenic lines (NIL). Next, they set about investigating how these QTLs influence HFN. "Some QTLs don't have any effect in some years, but will make a big ►

Unlike disease resistance, if you can breed in a better HFN, that'll last forever, points out Cristobal Uauy.

difference to HFN under certain conditions."

The lines have now been grown for a number of seasons in a range of conditions. They've also been subjected to misting during grain fill, where they're effectively lightly irrigated under a sprinkler system to mimic the conditions that would bring on sprouting.

"One of our QTLs is really mysterious — both NILs maintain their HFN if irrigated throughout grain fill. But if conditions are dry, with just a burst of moisture close to harvest, the HFN drops considerably in the susceptible NIL, but is maintained in the resistant line. With another QTL, there's no apparent difference in sprouting between the resistant and susceptible lines, but there's a big difference in HFN."

As well as looking to evaluate more about how each QTL behaves, the team is testing lines with 'stacked' QTLs, to see if they complement or conflict. Within the QTLs, they're also zeroing in on the specific genes responsible.

Smaller segments

"We've taken each QTL and sliced it up into around 90 smaller segments. With DNA markers we can monitor the size of the resistant QTL in each one of these lines. We want to see whether it's a progression — that they'll express the trait more or less, depending on how much of the QTL segment is in the genome — or whether it's black and white, meaning that a single gene within this segment provides the HFN resistance."

While this material is still being investigated in the field, initial results are in from glasshouse studies, and it's looking very exciting, says Cristobal Uauy. "We're seeing values that are sufficiently black and white to validate that some of these lines do carry resistance to the same extent as the original QTLs. In scientific terms, this is beautiful. It's early days and we need to keep slicing, but we are honing in on specific genes that protect UK varieties."

A clearer understanding of the genetics behind grain protein production is just one of the outcomes of a three-year project, led by Rothamsted Research. "There's a clear trade-off between yield and protein," explains Prof Peter Shewry of Rothamsted.

"As the yield increases, grain protein falls, and if plotted on a chart, most varieties fall on a straight line. But some perform above the line — perhaps by making better use of the nitrogen provided. We wanted to determine how these varieties achieved this by identifying differences which aren't related to the overall N response."

The project focused on six varieties — Cordiale, Istabraq, Hereward, Malacca, Marksman and Xi19 — which were grown at a number of locations using three different N regimes, and the relationship between grain N and grain yield was analysed. The effect of grain N on gene expression profiles was also determined, to see which genes were contributing towards the higher grain protein.

"We narrowed it down to less than 30 genes associated with building higher protein in the grain, but which were not simply responding to overall N levels," reports Peter Shewry.

"In addition, we've identified genes that are particularly responsive to N, including a novel family of gluten protein (gliadin) genes." Such proteins contribute to the gluten network, which gives dough the

Wheat has been subjected to misting during grain fill to mimic the conditions that would bring on sprouting.

Consistent quality needed for sustainable wheats

Varieties that consistently reach the required quality criteria are essential for the UK's millers and bakers, according to Mark Charlton of Allied Technical Centre.

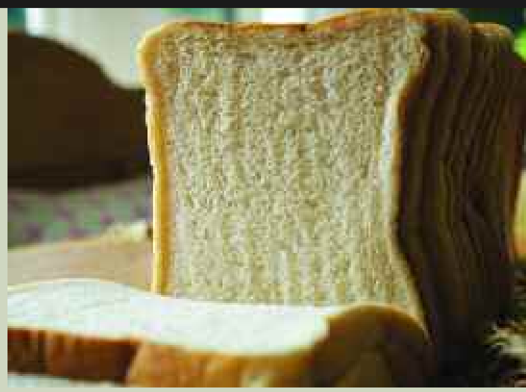
"We need a certain level of protein for flour to be baked into bread and other bakery products, but the key is the functionality of that protein," he explains. "There are certain varieties that are universally accepted, and millers can then work within tolerances.

"If the HFN dips below a certain level, you get too much enzyme activity in the flour. This leads to the breakdown of starch and results in sticky dough that doesn't form a good, structured loaf. While certain varieties have the genetic capability to maintain their HFN, a lot comes down to how they perform at harvest, and conditions at that time."

Consistency is a key factor, he points out. "Millers are working with a natural product, but need to produce a homogenous material, so inconsistency presents real challenges for us.

Recent research projects have led to a real step forward in understanding how the genetics of a variety is linked to these quality criteria, he believes. "Researchers have been piecing together a gene map that associates quality traits to genes which can be used when breeding new varieties. They still have the challenges of making sure a variety performs in the field, but if they can select lines based on known genetic markers, the chances of bringing consistent, high performing varieties to the market are greater.

"Ultimately, that means millers can have more confidence in the material they source, growers can deliver to the contract specifications and this will make UK quality wheats more sustainable," concludes Mark Charlton.



If the HFN dips below a certain level, this results in sticky dough that doesn't form a good, structured loaf.

elasticity required by bakers, he explains.

"Now that we've identified genes that are related to total grain protein and are particularly responsive to N, we can explore what they do in more detail. This may allow plant breeders to select for them in varieties."

This could lead to varieties that build higher protein at the same grain N, he says, as well as more stable levels of protein. "We're also looking to develop types of wheat suitable for bread-making at lower protein levels, including lines with greater stability. This will allow millers to reduce their requirements for grain protein content.

"In fact, some end users already work closely with the supply chain and can accept lower protein levels, without compromising dough quality. If we can focus on the specific qualities millers are looking for, we can develop suitable varieties that don't require high protein levels."

With vast amounts of data generated through the project, its analysis has been developed and improved, points out Dhan Bhandari, helping researchers refine how the information is interpreted. "We have very good quality data, but we now also have the means to distinguish the effects of different N levels and growing conditions on varieties very easily," he concludes.

Peter Shewry is keen to know how some varieties maintain their protein at higher yields.

Protein drop

But what about the best route for getting this N into the grain? Growers still face the problem that protein levels drop as yields increase, and tailoring N inputs can be something of a lottery, says Dr Richard Weightman of ADAS, who's been leading research in this area.

"At today's higher yields, you need N inputs close to the maximum allowed under NVZ regulations. So many growers choose to put on a late foliar urea to achieve the required grain protein, but don't have any way to test whether this will actually be worth applying."

The key would be to find a test a grower could perform on the crop, before the latest application date for foliar urea, that would predict what the grain protein level was likely to be. This was the focus of a three-and-a-half year study that ended in Sept 2010.

"To start with, we weren't even sure whether this was something that could be measured reliably and consistently without drying the sample first. But we developed a model and a test that was practical — we found you needed a grain N of 1.9% at the milky-ripe stage to achieve a grain protein level of 13%. The challenge then was to develop this into a practical service."

ADAS has tested a practical real-time service to predict the need for foliar urea this year with members of Camgrain, and the results look promising, says Richard Weightman.

The test involves using a lab-based machine, and results need to be turned around quickly to inform the decision over whether to apply the late N. "We've been aiming for a result that would be a bit like a spray recommendation, but there are big errors in sampling and variability — so it can be hard to show a benefit from taking the reading."

This was the case if performed on one individual field, researchers found. "But if the sample was part of a number taken over the farm or over the region, it gave a very good indication of whether to make the application," notes Richard Weightman.

The involvement of grain co-operatives Fengrain and Camgrain during the HGCA project provided this, as well as a means of gathering and testing the samples. With funding from a Sainsbury's research and development grant, ADAS has tested a practical real-time service this year with 15 members of the Camgrain wheat development group over 60 fields, and the results look promising.

"We'd found that you could raise grain protein by as much as 0.7%, so if your predicted level was only around 11.3% for example, there'd be no point making an application and the grower could then treat the crop as a feed crop. Equally, if the prediction came out above 13%, an application wouldn't be necessary. There was a relatively small window in the middle where foliar urea was worthwhile."

More targeted use of foliar urea and accurate prediction of grain protein in collaboration with the grain co-op mean end-user requirements can be achieved more easily, he points out. "There's also a bit of in-field variability to take into account, so we're looking at how we can use the results in conjunction with spectral images of fields to create spatially variable field recommendations." ■

Research round-up

HGCA project 3659, a breeders' tool kit to improve Hagberg falling number (HFN) for the economic and environmental sustainability of UK wheat, runs from Nov 2010 to Oct 2014. Its ultimate aim is the development of new wheat varieties with increased and more stable HFN under variable weather conditions. Led by the John Innes Centre, with partners KWS, Limagrain, RAGT, and Lantmännen SW Seeds, its total cost is £1,340,000, with £120,000 funded by HGCA.

HGCA project 3409, Sustainability of UK-grown wheat for bread-making, ran from Oct 2009 to Sept 2012. Its aim was to define the genetic and agronomic routes to achieve good quality bread-making wheats in the UK at lower N input levels. Led by Rothamsted Research, with partners Campden BRI, Limagrain, RAGT, Syngenta, KWS, ATC, ADM, Heygates and Rank Hovis, its total cost was £1,064,000, with £190,000 funded by HGCA (plus an extra £10,000 in-kind funding).

HGCA project 3211, Predicting grain protein in order to assure bread-making quality and minimise diffuse pollution from wheat production, ran from April 2007 to Sept 2010. Its aim was to improve the efficiency of decision making when applying nitrogen fertiliser to milling wheat, using a crop-modelling approach combined with NIR sensing of crop nitrogen content. Led by ADAS, with partners Bruker Optics, Camgrain, Campden BRI, Fengrain and Heygates, its total cost was £656,000, with £110,005 funded by HGCA.