The threshold before the leap?

> "It's about making use of a technology that can accelerate how we bring our discoveries to the field, and the shorter timescales will bring in farmers."

Innovation
Future wheats

Two decades of pioneering plant science in the UK have brought into the field staggering advances in genetics. Now it's time for growers to get involved. *CPM* took a tour to open a window on a world of wonders.

By Tom Allen-Stevens

They look like little more than knots of root and shoot matter held in place in the agar of a petri dish as Dr Sadiye Hayta holds them up towards the web cam for Prof Cristobal Uauy to view. But these represent something of a breakthrough for the team at the John Innes Centre (JIC) working on Designing Future Wheat (DFW), and a virtual high five passes between the two colleagues.

"What we're looking to establish is genotype independence," explains Cristobal, who's joined the meeting via Zoom web conference. "We've been working with a genetic trait of *Triticum polonicum*, a durum wheat that has unusually long glumes and very long grains. We're aiming to introduce the trait into the hexaploid varieties Paragon and Fielder and another durum wheat, Kronos, through gene-editing."

This process is currently genotype dependent which limits the ability of scientists to use the new plant-breeding technique (NPBT) to introduce new traits into commercial, elite lines, particularly those that no longer exist in modern varieties. "We've been working on a new technique developed by colleagues at University of California Davis for a year and a half, and this represents the first sign of success, which could be revolutionary — it'll mean we can turn around near isogenic lines (NILs) of elite varieties in just 2-3 years," he enthuses.

Lengthy process

This is currently a process that takes at least eight years through conventional plant breeding, even when using modern gene markers and doubled haploid techniques, he explains. Once a trait is identified, the wheat type that has it is crossed with an elite line that would benefit from it. Successive generations are then crossed and screened to sift out hundreds of other undesirable traits that have come over in the cross, to reach the NIL — the commercial line with just the new introduction.

"It can take 10-15 years to bring in more unusual and exotic traits, particularly if it comes from landrace or wild varieties," Cristobal continues. "It's a process that's not viable for traits that are less commercially valuable which is why current UK wheat varieties are bred from a very narrow gene pool. Breeders are simply shuffling the pack, but DFW aims to bring new cards into play, which could be transformative."

DFW is a BBSRC-funded programme spanning eight UK research institutes and universities. A five-year series of four work packages that started in 2017, it follows on from the Wheat Institute Strategic Programme (WISP) and involves more than 25 groups of scientists across Rothamsted Research, JIC and Earlham Institute, with additional contributions from NIAB, the European Bioinformatics Institute (EBI), and the Universities of Bristol and Nottingham.

Key features of DFW arethat all the knowledge is made publicly available and UK commercial breeders are involved from the outset.

Driving forward this public-funded search for the new wheat germplasm containing the next generation of key traits is the projection that the world will need to produce 60% more wheat by 2050 to meet global demand. But yields in the UK have plateaued and it takes 15-20 years for current research to improve wheat varieties grown in farmers' fields.

That's encouraged a deep delve into the very fundamentals of the wheat genome — research credited as unique to the UK, and now accelerating as new techniques evolve. Some of the world's foremost plant scientists have explored landrace varieties,germplasm previously never included in wheat-breeding programmes, and even returned to the original cross that 10,000 years ago formed today's hexaploid wheat (*Triticum aestivum*) from tetraploid emmer wheat and goat grass.

Now, some 12 years after WISP first started, the first of the most promising finds from this pre-breeding community are

Scientists at JIC are looking to establish genotype independence, introducing novel traits directly into elite lines.

What is a GMO?

This lies at the heart of the debate on NPBTs. Under EU law, any process that involves the introduction of foreign DNA or RNA classes the resulting product as GMO. But there are a number of ways through which a plant can undergo a genetic change:

Transgenesis is where DNA from another species has successfully been combined into the genome of the host plant. This confers a new trait, such as herbicide tolerance or longer shelf life. These organisms are universally classified as GM.

Cisgenesis is a term used by some scientists who argue for lighter-touch regulation, where DNA is artificially transferred between organisms of the same species, such as from a wild relative to an elite potato variety to confer blight resistance. In Europe at least this is still classified as GM as nucleic acid sequences must be isolated and introduced using the same technologies that are used to produce transgenic organisms.

Mutagenesis is a change or edit in the plant genome that confers a new trait. Such mutations occur naturally every day, when a plant comes under stress, for example, or it can be induced through human intervention. A small change in the genome may switch off the activity of a particular gene which allows or inhibits a property, and it's these phenotypical changes breeders have sought out for generations to progress their lines.

Is it natural?

For decades, scientists have induced mutagenesis to bring about new traits, using chemicals or radiation, and the Clearfield trait is an example. More recently, more precise gene-editing techniques such as CRISPR-Cas9 have been introduced. CRISPRs are short RNA sequences introduced into the host plant that recognise a specific stretch of genetic code. Cas9 enzymes partner these sequences and cut the host DNA at specific locations.

Scientists argue the genetic edits they make are simply a precise and predictable way of inducing a change that could have occurred naturally.

The cell tries to repair the damage, and that's when the mutation occurs. By using different enzymes and techniques, researchers can deactivate or alter — edit — specific parts of the genome, thereby conferring traits. Scientists argue the genetic edits are simply a precise and predictable way of inducing a change that could have occurred naturally. But under EU law, the introduction of foreign RNA, even though the RNA is not present in the final plant material, classes current forms of CRISPR as GMO.

making their way from the lab to the field. From tiny first generation plots formed from one ear of wheat to replicated field trials of NILs, covering several square metres, novel material of every shape and form can be found in the fields of research institutes and universities across the UK and even in commercial breeders' plots among their elite lines.

"Some of the most exciting traits have been found in the Watkins collection," notes crop physiologist at Rothamsted Research Andrew Riche. He's been involved in some of the high throughput phenotyping — spotting the varietal differences in the field ▸

The first of the most promising finds from the pre-breeding community are making their way from the lab to the field.

▸ that have come about from the thousands of crosses made. He uses drones to assess traits such as nitrogen use efficiency, crop development and biomass yield as well as Rothamsted's impressive Field Scanalyzer. This comprises a gantry with a motorised measuring platform festooned with sensors that monitor crops to a high degree of resolution and reproducibility.

"A E Watkins was a British botanist who collected around 1000 wheat cultivars from around the world during the 1930s, now held at JIC," he explains. "These have been genotyped and there are 120 lines that represent the overall genetic diversity of the collection — far greater than the diversity currently used by European and UK breeders."

These have been back-crossed into Paragon and, along with the many other novel crosses coming through from the DFW partners, the resulting NILs enter the Academic Toolkit. This operates a bit like a Recommended List for breeders, says Andrew. "Lines are selected each year to go forward to the Breeders Toolkit where material is made available to breeders along with genetic markers."

There are 16 lines going forward from the 2020 harvest of the trial plots, with this the third year the system has been operating. "There's talk of stepping up the programme, and it's not just about yield — there are some very interesting disease and pest resistances being studied as well as grain quality and nutritional benefits, such as improved zinc and iron content. The involvement of breeders has been pivotal in bringing this material into the field," he notes.

But this move to the field is posing a problem. Some of the material has been developed using NBPTs, specifically gene-editing, which have been classed as genetically modified organisms (GMOs) by the EU. This restricts their use and how they're released to the environment. Most other nations, including the US and China, view NPBTs as conventional, however, and most UK plant scientists agree. Defra

Be among the first to grow slug-resistant wheat

Farmers are being given the opportunity to grow a wheat variety scientists believe may be resistant to slugs. "We decided to screen some of the novel Watkins material for slug resistance as this was identified as a priority," says Prof Simon Griffiths of JIC who leads one of the DFW work packages.

JIC entomologists set up feeding experiments — a set of choice chambers that allows slugs to choose at random varieties they would like to graze and those they prefer to avoid. "One wheat was consistently spurned — Watkins 788," he reports.

This was taken into small plot field trials in 2015/16 and the results confirmed the initial findings. But the research hasn't progressed any further. "We don't know yet whether this wheat truly resists slugs or whether they'd still eat it in a field situation where there's no other choice."

So larger, field-scale plots are needed, which is where grower involvement comes in. "We're looking for farmers who take part in on-farm trials who'll plant an area of around 0.4ha of Watkins 788 in a field prone to slugs as part of a scientific study — we think that will be an area sufficient to provide the optimal foraging distance to study the slugs' behaviour," says Simon.

He cautions that the wheat is an old variety that would perform fairly poorly in every other respect next to a modern wheat. "But if it truly resists slugs, that will be a very valuable trait and we'll want to do some work to identify the QTLs." These quantitative trait loci allow researchers to link phenotypes to specific genetic regions. It's the first step in capturing a trait that can subsequently be bred into commercial lines.

● The slug trial would be run as a Rothamsted FarmInn farmer-led trial and wouldn't be planted until autumn 2021 to allow enough time to multiply up the seed required. Those interested in taking part should contact the British On-Farm Innovation Network (BOFIN) through tom@cpm-magazine.co.uk by 30 Sept 2020.

The mottling on the leaf of this diploid variety is where the plant protects itself from attack by a fungus through stopping the infection.

Lines of interest

The plots at Rothamsted in Herts illustrate the raw state some of the DFW wheats are in. Close to harvest, Prof Kim Hammond-Kosack conducts a tour for *CPM*, pointing out lines of particular interest.

"This is where we're selecting lines that show particular promise for disease resistance. Some material is in the field for the first time, and we just have an ear row, derived from one ear, while others are more substantial 2m wide plots of six rows."

Recent successes through the plots include the discovery of a very robust resistance to yellow rust. "We first identified the lines as far back as 2007, and they've withstood all the changes in races that have taken place since.

Kim Hammond-Kosack has been selecting lines that show particular promise for disease resistance.

The resulting NILs are currently in the Academic Toolkit and also in breeders' observation plots."

She comes to one line that appears to have a fine mottling on the leaves but it's completely clear of disease. "This is *Triticum monococcum*, einkorn wheat, a diploid," she explains. "The mottling is where the leaf protects itself from attack by a fungus through stopping the infection. It's resistant to septoria, yellow rust and brown rust."

The trait was crossed into Kronos, a tetraploid, and finally to hexaploid Paragon. "It took us ten years and three strategies, but we finally succeeded and now we can study this novel trait in more detail," notes Kim.

has always maintained that "gene-edited organisms should not be regulated as GMOs if the changes to their DNA could have occurred naturally or through traditional breeding methods".

The Government's now announced there'll be a consultation this autumn on whether to diverge from EU policy on gene-editing and much may hinge on the feelings of UK growers if NBPTs are ever to be planted in their fields. But a recent survey undertaken by the Gene-Editing for Environmental and Crop Improvement initiative showed as much as a third of even the more progressive arable farmers feel they don't know enough about NBPTs to have an informed opinion.

Promising germplasm

And that's why these knots of promising germplasm hang in the balance. So too does much of the research that sits behind them, captured into wheat plants of every description in JIC's light rooms and glasshouses at Norwich Science Park. The institute is at the forefront of wheat research globally and one of its landmark successes has been the development of a GM wheat with a natural high-iron content in its endosperm.

"The aim is to address iron deficiency anaemia, a significant global health problem," explains senior geneticist Prof Wendy Harwood. "Tests on the newly developed variety grown for the first time in the field last year showed that the grain contained double the amount of iron in its white flour fraction."

She points to where the trial took place, inside a high-fenced, locked enclosure. This is one of only two field locations in the UK specially adapted for ensuring the controversial field trials of GMOs can continue within the current regulations and without disturbance by anti-GM activists.

"We don't get much bother from activists these days, but it was COVID-19 that upset trials due to take place this year. Lockdown occurred just as colleagues were about to set out the trial, although we have continued with the research under glass."

GMO field trials don't require such high security, but they must be conducted to a level of regulation that's currently above what's required for conventional crops (see panel on p72). That makes them prohibitively expensive or burdensome for many involved in wheat research, says Wendy.

"It means we can't involve breeders in any elements of our research that involve NPBTs, and we've learned through DFW how important it is for commercial pull through to have this engagement right from the start.

But scientifically, gene-edited material doesn't need to be regulated differently from conventionally-bred plants."

Cristobal explains that the mutations he and his team generate occur every day in the field and do not involve the introduction of foreign DNA. "The difference with a natural mutation or one that is chemical-induced is that our technique is precise — we know exactly where in the wheat genome the change has occurred. Typically, the technique just a few letters of DNA code in a plant that has several billion. There have been concerns for so-called 'off-target' effects, but we have a system to check for these, and a wheat seed typically has around 90 natural mutations with respect to its mother plant anyway," he notes.

Through marker-assisted breeding,

Despite their clear potential, gene-edited wheats are still stuck inside light rooms and glasshouses, and scientists can't involve breeders n the research.

scientists now know the locations in the wheat genome responsible for a specific trait. The way in which DFW relates the ►

phenotype — what's seen in the field — with the genotype now allows the potential for traits to be edited direct to a wheat genome without having to make a cross that brings with it undesired genetic changes.

"In just one process, gene-editing delivers as near to an isogenic line as you can get," he points out. "A farmer wouldn't be interested in growing a landrace variety no matter how good its disease resistance or size of its grain, nor would they grow Paragon with that trait introduced. But they would grow Skyfall with an improved grain or better yellow rust profile. That's why genotype independence is such a key point."

Scientists currently work with varieties such as spring-sown Paragon, Cadenza and Fielder — a US milling wheat — because their genomes have been fully mapped and their phenotypes heavily researched. This makes them suitable templates for novel traits when working with breeders. But Cristobal wants to work directly with commercial varieties and involve farmers as well as breeders in his research.

"Under the current regulatory regime, the UK is falling behind on gene-editing, although this is not about leading the world. It's about making use of a technology that can accelerate how we bring our discoveries to the field, and the shorter timescales will bring in farmers.

"Gene-editing is also relatively inexpensive if it's regulated as conventional breeding. So unlike GM, we don't need large multi-nationals to make a step change in how we grow wheat — there is potential for small-scale collaborations to bring forward niche ideas and innovations. This could not only lift the UK off its yield plateau, but provide solutions for the world for a more sustainable crop. We have the talent in plant science, breeding and farming to achieve this. All it needs is a science-based policy to govern its direction." ■

D stands for diversity and drought tolerance, scientists discover

For some it would be an undesirable misfit. But an ear of wheat that appears to have branched is a thing of fascination to Dr Phil Howell, senior research scientist at NIAB, Cambridge.

"The way these wheats are developing is multi-faceted and complex and this trait seems to be slowly stabilising in a few lines. It may have unwanted side effects, such as encouraging ergot. Or it may be transformative," he says.

The team at NIAB have been working primarily with synthetic wheats. These have been bred by returning to the original cross of tetraploid wild emmer with wild goat grass to form the modern hexaploid wheat. "We're interested in capturing the D genome," he explains.

"The diversity in modern wheats has been largely lost because it's dominated by the A and B genomes. We bred 50 synthetic lines that captured the whole of the D genome and have been exploring what properties it brings to wheat. We're looking for the phenotype of resilience."

The wheats have been backcrossed into Robigus and Paragon, and it's these accessions that are now out in the field, in plots from single ear rows to replicated plots looking at yield assessment.

One trait the team is looking at closely is flowering time. "We've identified lines with short or longer flowering times and those that flower early or late," notes NIAB director of genetics and breeding Prof Alison Bentley. "We now have markers identified to help breeders select for it."

This could be one of the traits related to drought tolerance, and some of the wheats have been through punishing trials to see how they fare. "Early flowering lines tend to do badly in most situations, but that can change depending on when the drought hits," reports Dr Robert Jackson, whose been assessing the plots.

Performance has varied considerably, he says, which is helping to identify traits such as root architecture and length of growing season

The branching is a sign of the novel traits introduced through the synthetic wheats that could be transformative.

that influence drought tolerance. "We're looking for types that perform well in normal conditions as well as under stress. Once we have the phenotypes, we can go searching for the genetic markers."

High fibre wheat set to improve nation's health

Researchers led by Rothamsted and JIC have applied some of the health benefits of wholemeal bread into the taste and texture of a white loaf. The discovery comes from identifying the wheat genes involved in controlling dietary fibre content in flour.

"We've been working with the JIC to pin down QTLs for high-fibre traits," explains Rothamsted's Dr Alison Lovegrove. "It's not a trait UK breeders select for, so isn't found in elite varieties."

The team found the trait in a Chinese variety, Yumai 34, but this unusual type doesn't grow well in Europe and little is known about its genome. "We successfully crossed the high fibre trait into several other varieties. This allowed us to narrow down where in its genome the genes for high fibre are."

The team has now identified two molecular markers and the wheats are going into the Academic Toolkit for evaluation this September. "Around 80% of the population choose white bread, so white flour that is naturally high in fibre would make a real difference to the health of the nation, as well as reducing the incidence of Type 2 Diabetes. We believe the trait doesn't carry a yield penalty, so hope that it will one day become ubiquitous in quality wheat varieties," says Alison.

The achievement is just one of many nutritional benefits researchers say they have identified through exploring the diversity of the wheat germplasm across the DFW programme.

Alison Lovegrove hopes the high fibre trait will one day be ubiquitous in UK quality wheats.

Bread with a high natural iron and zinc content is also on its way, following the discovery of the trait in Watkins lines that have now been crossed with Paragon.