



Wheat through the ages

Exploring the genome

Modern wheat breeding is constantly advancing, bringing yield and agronomic improvements to the table every year, but could looking back at old varieties bring something new to the table? CPM explores how one scientific project is doing just that.

By Melanie Jenkins

For at least 8000 years, wheat has been a staple food source across large swathes of the world and is now one of the most common crops grown. But the rising human population is demanding more of this grain than ever before.

Overcoming some of the increasing pressures on growing wheat and producing enough to feed the world is a task that breeders are tirelessly working on, it's something that genomic sequencing is helping with. "Wheat is a fascinating crop," according to RAGT's Dr Richard Summers. "The crop came about as a natural hybridisation of three wild grasses, so from a breeding perspective, there's the potential to access useful genes in ancient bread wheat that are hexaploid, meaning they have the genetic composition of the three grasses.

"When this happens, you can go back into old collections of wheats to look for new traits and you can even go back to the wild

grasses that formed this hybridisation. There are some constraints, but the amazing thing is that between wheat and the related grasses, you can intercross them without the use of genetic engineering."

Adapted for our systems

So there's a question about whether there are genes that have been left behind from modern varieties, says Richard. "Over the past 100 years plant breeders have bred varieties adapted for our farming systems. This doesn't mean we've been breeding varieties that aren't resilient, but that modern varieties are bred to be grown under higher fertilisation and to produce higher yields. Although some might say these varieties require more husbandry to reach their potential, I believe that modern varieties grown in lower fertilisation situations and in poorer conditions, would still outyield some of the ancient types."

In terms of what breeders have been able to achieve, they've managed to change the idiomorph of the crop so that more of the above ground matter ends up as grain that can be harvested — the harvest index, he explains. "The thrust through wheat breeding is to produce high yields and this has a huge socio-economic impact."

One of the issues with some of the current constraints is whether breeders can bring in better disease or heat stress resistance, he points out. "When we look at our current cultivated wheats, we won't have enough diversity to cope with these challenges. So by going back and looking at old collections we might be able to identify where bottlenecks of selection have left some useful genes behind."

This won't be a magic bullet, stresses



“ Breeders live for diversity to be able to advance breeding. ”

Richard. "We won't suddenly find a gene that will allow wheat to cope with a 2°C rise in global temperatures in an archive. ▶



According to Richard Summers, by looking at old collections of wheat we might be able to identify where bottlenecks of selection have left some useful genes behind.

Wheat through the ages



For thousands of years wheat has been a staple food source across large swathes of the world and is now one of the most common crops grown.

► However, looking at a collection of wheats that were growing in warmer environments will likely include some useful genetic material, that if we can uncover it, could be useful today.”

The problem is how to uncover this material. “You either have to know something about the genes you’re trying to look for, or about the characteristics which are under genetic control that will provide some of the things you’re looking for. So how do we identify what is a beneficial gene and then how do we find it? Looking at historical collections we can see what variability is there, but at the same time we have to be informed about the current genomic studies.”

Sequencing allows for the identification of genes and can help to speed up and

improve breeding, explains Dr Matt Clark of the Natural History Museum (NHM).

“There are two ways of knowing whether a plant is going to be potentially useful: one is to look at it and the other is to look at its DNA,” he says. “When looking at a plant, it’s not always obvious what’s going on because with a trait like yield there may be thousands of different genes contributing to this and some will be affected by the environmental and seasons. Whereas looking at genetics provides a more absolute answer and the ability to pick genes.

“Wheat yields are already increasing by 1-3% per year through various breeding efforts, but if you look at the DNA it’s possible to see where each gene adds to the overall yield. This would be hard to see in the field but genetically it can be picked

up and allows breeders to stack these genes in a new variety.”

Matt, who is the lead researcher on the NHM Wheat Through the Ages project, was one of the team tasked with originally sequencing the wheat genome while at the Earlham Institute. “This was a complex and expensive undertaking because there are three genomes, so to make it feasible he used short-reads, where the genome is broken into small fragments before being sequenced. But at 100 bases long it was a struggle to tell the difference between the A, B and the D genomes.”

First wheat genome

However, this allowed him to sequence and assemble one of the first wheat genomes.

“We were due to sequence two of wheat’s 21 chromosomes but in the end sequenced the entire genome,” he explains. “We also created a tilling population, which involves deliberately mutating an organism to make changes to the DNA, and then made probes for each of the chromosomes, so that instead of sequencing the whole 15-17 gigabase genome, we could just sequence the 0.1 gigabase that make genes. This was then sequenced for each of the different wheats that were mutated, and we could subsequently work out what the mutations were.

“This was put into a database of all the genes and different mutations. Because we then knew a sequence, we could predict what a mutation is likely to do.”

This then led to sequencing NIAB’s Elite Magic population, which is an eight-parent population, says Matt. “This is where eight elites from the AHDB Recommended List were crossed, and it covers 90% of the genetic diversity in the UK wheat germplasm from the late 1980s. The aim was to get around a thousand offspring which have DNA from each of these parents mixed up in different combinations which has been used to do trait analysis and to map different genes.”

To gain an understanding of wheat genetics from outside the UK, the researchers brought in two varieties that were quite different — a heat and a drought resistant variety which are used in programmes for the developing world — from the International Maize and Wheat Improvement Center (CIMMYT).

From here, this led to involvement with the 10+ Wheat Genomes Project, which helped pave the way for further research when it sequenced the genome of 16 wheat varieties from breeding programmes around the world, identifying how much ►



Researchers at the Natural History Museum, Kew Gardens and the Hebrew University of Jerusalem have been digitising their wheat archives to see how the wheat genome has changed.

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Wheat through the ages



There's potential to access useful genes in ancient bread wheat which are hexaploid, meaning they have the genetic composition of three grasses.

▶ variation there is in wheat.

"We've gone on to skim sequence DNA from 92 crop wild relatives, which were chosen based on a study from CIMMYT, which had genotyped about 100,000 different wheat varieties included 8-9000 wild relatives. This has given us an idea of what is going on in wheat," explains Matt.

Now, researchers at the Natural History Museum, Kew Gardens and the Hebrew University of Jerusalem have been digitising their wheat archives to see how the genome has changed and there are hopes to identify genes that could help breeders in their task to produce more resilient varieties. The Wheat Through the Ages project involves sifting through 13,000 specimens from the NHM and 7-8000 more from Kew, plus others from Israel of the Triticeae family, which includes wheat, barley, rye and wild

crop relatives, which dates back 300 years. "The aim is identifying how wheat has changed over time," says Matt.

The project is still in the early stages of looking at historical varieties with the first round of analysis currently being conducted. "There's a lot to do with around 200 varieties that we now have some level of sequencing on," he explains.

"We're interested in identifying genetics we might have lost such as quality traits that it might be useful to bring back into modern breeding, and also if historical varieties had high yields with fewer inputs than what we've become accustomed to using.

"There are multiple genetic pathways that could allow for this," says Matt. "Firstly, it's about having a better symbiotic relationship with the microbes in the soil — the bacteria and fungi — which the plant

is supplying with sugar in exchange for nutrients. But in a modern field environment, there'll be a lot of nutrients available to a plant, so why would a plant supply its sugar to the bacteria when it's not short of anything. Because of this, we're interested in looking at whether this has been selected out because it's become less useful."

Beyond this, Matt and the team are keen to explore the capabilities of the pumps in different varieties which move the nutrients from the soil to the rest of the plant, as well as the efficiency of use and the recycling of nutrients such as nitrogen. "We may well also come across resistances or abilities to tackle marginal situations and it would be nice to find varieties that are more efficient at using fertiliser as this is both beneficial for farmers and the environment."

One challenge with trying to incorporate useful diversity comes in the form of payoff, says Richard. "For example, if material was discovered that appeared to have better water use efficiency and resistance to drought — which could a mix of all sorts of things such a different leaf or root structures — but it comes with a 10% yield hit. It might be that one of the reasons this hypothetical variety survives is because it doesn't respire and grow as strongly as another variety would with unlimited water. But this in itself could be useful as it could provide a crop that grows in a more challenging environment."

Disease resistant genes also pose their own issues related to durability, he highlights. "If we find a genetically inherited source of resistance in an ancient wheat, we might be able to transfer it but there's no

Sequencing

So just how is the team at the NHM digitising and sequencing its historical collection? It's a complicated process because the material in the archive is degraded because it's older, meaning the DNA is broken in smaller pieces, explains the Natural History Museum's Dr Matt Clark. "First of all we have to convince the curators that we're doing something valuable with their samples.

"We've tried using different tissue types including the leaves, roots and awns, but if you cut a leaf off it's fairly obvious, but you can get away with cutting the end of an awn fairly easily.

"We disrupt the tissue physically by shaking it up with steel balls which turns it into dust, and then we extract the DNA using a mixture which protects it from degrading and then bind it to magnetic beads," explains Matt. "There are

specialist techniques for making the libraries where we put adapters onto the DNA to deal with these short damage parts of DNA."

The DNA is around 50 base pairs in size once sequenced with Illumina technology, but if this was done with a modern plant the team would be able to take 300-500 base pairs. "We can sequence on a larger scale if we use a different platform such as a nanopore sequencer where we could take 40,000 base pairs."

From here, the samples are processed and put together in a sequencing run. "Each library from every sample has a DNA barcode that indicates which sample that piece of DNA belongs to. It's more affordable to run all of the samples together but the barcode allows us to sort them out afterwards. We then map the DNA to the different genomes and can



Sequencing historical wheat varieties is a complex process due to the material in the archive being degraded, meaning the DNA is broken in smaller pieces.

determine how much has mapped and how complex the library is. From this point, the team is able to identify the differences, or polymorphisms, between the reference wheat genome and what they've sequenced."

guarantee that it'll last more than a few years before the disease responds and the gene is overcome. However, it could provide another source of diversity to replace any resistant genes that are being broken. And some work being undertaken currently is actually looking into which pathways may be more or less durable."

If the team does identify useful traits, it'll take some time before the industry sees these come through in new varieties. "Material that is coming from the Magic population is so close to what's already being grown that it can come through in new varieties quite quickly, but it would take longer to incorporate traits from the historical material," explains Matt. "If we did identify a trait in one of these varieties, we would also have to find a specimen that's alive or we'd be using some form of gene editing technology — and this could depend on the legislation and the market."

It might be the case that traits are incorporated into varieties suited to other regions, such as India, South or Central America and China. "It'll just depend on the nature of what we discover. But if, for example, we gained a fundamental understanding of fertiliser use or increasing the efficiency of nitrogen use, this is the type of thing we could edit into perhaps all varieties of wheat."

The projects preceding Wheat Through the Ages have already proved that this type of research can help solve issues and answer questions. "Through the work on the Magic population, genome sequencing has helped resolve questions about resistances and different traits and how they're actually made," he says.

"And the Crop Wild Relatives project has been used to try and understand introgression. A lot of the ways traits are put into modern varieties is through breeding



Breeders are already using marker assisted trait selection a lot, but mapped genomes are providing markers that are really closely associated with the trait they're after.



The 10+ Wheat Genomes Project helped pave the way for further research when it sequenced the genome of 16 wheat varieties from breeding programmes around the world.

in resistance genes — typically biological resistance for a pathogen or an abiotic one to difficult environmental conditions. Some of this work has already been done, and we know the traits are there and roughly where they are on the chromosome but with the sequence of the progenitor, we can work out exactly the area of the chromosome, so how many megabases — millions of base pairs — have actually come in from this wild relative. If you know the gene you're after, you could possibly try to find varieties where the other bits of genes have been lost.

Design markers

"There can be hundreds of genes in one area in that area, but might only be one or two you're interested in and the rest might be decreasing the agronomy of the plant. So we could use this work to design markers to swap the unwanted areas of chromosomes out for elite versions, for example," he details.

It'll be some time before the work of the Wheat Through the Ages project can be used by breeders but the path to it providing another tool is already outlined. "Breeders are already using marker assisted selection a lot, but the genomes are probably providing markers that are really closely associated with the trait they're after. Previously, the worry was that breeders were using a good marker, but it could still be a million base pairs away from where the gene is actually located.

"So at times the marker would indicate a good line, but when the variety goes into the field it becomes apparent that it doesn't have the trait it's supposed to have because there's a crossover between the

gene and the marker. So having the complete genomes and being able to pick a marker that's right on top of the trait is really useful."

Another aspect breeders have been able to advance is their understanding of how disease resistance works and how the genes involved can be stacked. But yields are more complex and it takes a long time to line up in a variety, as they aren't single dominance traits. Sometimes a small percentage gain from a gene will only work when it interacts with another gene, which can require an understanding of the whole network."

Whether the project unearths traits that can be bred into modern varieties or not, it's creating a useful resource for breeders, says Richard. "RAGT has genomic maps of our modern varieties but our insight isn't complete. However, if sequencing is being made from old varieties then we can do two things, the first is seeing the difference between ancient and modern wheats. And secondly, in certain cases, if we know the area of the genome in modern wheats which is involved in disease, yield or tolerances of various stresses we can predict what these are likely to be like if we grow them."

He points out that the industry can sometimes indicate that there has been a loss of diversity in plant breeding. "We talk about a loss of genetic diversity as a bad thing, but some of this is from attributes such as weak straw or excess height. Breeders live for diversity to be able to advance breeding. So any research project that gives us access to novel and useful diversity is a good thing that should be encouraged." ■