GENETIC

LOTTERY

Complexity makes for daunting task for breeders

BY GORD LEATHERS

Want to get a sense of just how difficult it is to work with wheat as a plant breeder? Just take a look at one of the most difficult plant diseases to hit the crop in recent times.

Fusarium headblight hit the Canadian wheat crop back in the mid 1980s and researchers responded quickly, putting a breeding program for fusarium resistance into high gear.

But results were slow to come. It's only in the summer of 2009, that a hard red spring wheat with a resistance rating of "good" to fusarium will hit farmers' fields.

While breeders were working overtime, more than 20 years passed before the first tangible results were available — and according to those who work with wheat as breeders, that's about par for the course.

That's because anyway you slice it, wheat breeding is a daunting task.

For one thing, it has a huge and complex genome so playing the genetic roulette game with wheat involves really big numbers.

Secondly, to take the genetic journey from first cross to farm field requires a high degree of intellectual cross-pollination between breeders, plant pathologists, entomologists and biochemists all working together to understand the pests attacking it and manipulate this unwieldy beast to build resistance.

It simply takes time and it takes a lot of highly qualified personnel — but why is wheat so unwieldy?

The answer lies in the extremely complex genetic blueprint of the wheat plant. Without getting into too much gory detail, some field crops, like barley, are relatively simple diploids, which is a fancy way of saying that they get one set of chromosomes from each parent.

Flowering plants, however, often manage to capture chromosomes from other species that find their way into the mix, not an unlikely event when you consider that plant matings happen when insects visit, rain falls or wind blows. Instead of being diploid, up to half of the 300 thousand species of known flowering plants are polyploid, meaning they contain more than two parent lines.

Diploid organisms are what most people understand best — for example, humans are diploids.

Ron DePauw, a wheat breeder with Agriculture and Agri-Food Canada (AAFC) in Swift Current, says wheat has many more chromosomes for breeders to take into account.

"The wheat genome has more genetic base pairs than the human genome does," DePauw says. "It's what we call a hexaploid wheat, because it's made up of three different grasses."

What's this, you ask, three different grasses? Yes, it turns out that wheat is an unholy alliance of multiple parent lines — a complicated genetic stew that rose way back in antiquity that scientists, even now, are struggling to figure out.

The first contributor is Triticum boeticum, more commonly known as wild einkorn. Einkorn is a bunch grass that grows wild in through the Anatolian Peninsula of what is now Turkey and up the slopes of the Zagros Mountains of Iran where it's looked down into the valley of the Tigris and Euphrates Rivers since the twilight of the last ice age. It's also long been used as a food source, as it's been found in stone-age campsites excavated in the region.

Wild einkorn is the first of those three plants that express their heritage in that huge genome. It's the root stock, the genetic foundation of the world's bread box and wheat breeders refer to it as "Genome A."

But there are also two other ancient grains lounging around within the wheat genome. They're called genomes B and D and this is where the genetic fireworks begin.

We're not quite sure where the Genome B came from and there's still a lot of speculation about it — something that's guaranteed to set off the occasional spirited discussion when wheat breeders get together for a few barley sandwiches.

The third source, Genome D, has been identified as that of a goat grass, Triticum tauschii, which grew wild in the fields with the first cultivated wheat. Likely a natural crossing with the first cultivated wheat crops resulted in a stable offspring that was selected by the first farmers by the usual criteria such as improved yield or hardiness as well as a strange additional property that is the key to modern bread wheat. It's the goat grass that brought gluten into the seed and made leavened bread possible. No other grain can do that.

The important part to remember, though, is that somehow these three separate plant genomes have become incorporated into bread wheat. So now wheat breeders have one plant to work with that's genetically composed of three different species living together in the nucleus of every cell. Anita Brule-Babel, a wheat breeder at the University of Manitoba, says it's a small miracle that we have wheat to work with at all.

"Wheat is what we refer to as an allopolyploid — which means that the genes come from different species," she says. "It's a fluke of nature that we actually ended up with a wheat with three different genomes that's genetically stable."

That's because when different species combine, the best they can usually hope for is something like a mule — an only semi-viable organism that's sterile and lasts for a single generation. But occasionally one will work over the long term.

"And there goes Darwin again." Brule-Babel said. "When you're looking at millions and millions of plants crossing in a prehistoric wheat field, even though things like viable seeds have a low probability it's still possible that they can occur."

Consequently we wound up with bread wheat, a plant that's been reliably producing food for hungry mouths for thousands of years — and viable seeds to grow the next crop.

As well as being a minor miracle, wheat's also extremely complex, as a result. That's because basic genetics — if such a thing exists — states that the nucleus of the cell contains genes arranged in long stacks that we all know as chromosomes. Since we humans have two sets of chromosomes — one from each of our parents — we're one of the aforementioned diploids.

But wheat? It quickly gets a whole lot more complicated than that. The three genomes are all in there together. But they still stay together as three distinct genomes within one nucleus. Genes will shuffle within the genome but they're still kept within the genome. Genome A does not share with B or D.

Sounds a bit complicated? You're not mistaken. It is. And it's not about to get any simpler. Flowering plants such as wheat also already have an amazing capacity to stack whole chromosomes like cordwood and live with huge numbers of genes fighting for dominance in subgroups known as alleles.

When a gene wins dominance in that group of chromosomes the allele will affect a certain characteristic within that organization — hair colour, for example, in humans.

But then there's the issue of dominant and recessive traits — black hair versus red, for instance. The gene for black hair is dominant while its partner for red hair is recessive. A red headed child needs two genes for red hair, one from each parent. A gene for black hair paired with one for red delivers a black haired child. This makes redheads much rarer.

To make things more complicated, different traits are not just the result of different genes fighting for dominance on one allele. Some of them come about because of the interaction between different alleles on different chromosomes.

This complex mix is the way genetics works in any living creature — but the addition of the

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BALANCING ACT

As if the genetic lottery wasn't tough enough, there's also the issue of balancing the needs of the breeder's two most important customers — the farmers who grow the crop and the millers and bakers who buy the wheat and convert it into all the products like bread and flour for hungry consumers.

For farmers, the need is fairly straightforward: they want a disease resistant, quick maturing, reliable crop that will stand and deliver.

The miller or baker who buys the bread has a more complex set of requirements, however. They're looking for wheat that can fit within their large-scale processes that will perform reliably.

At the very core of it is gluten, a protein unique to wheat and this protein contributes something to baking so fundamental and so ancient that we take it completely for granted.

"It's the fact that you can make bread out of it." explains wheat breeder Gavin Humphreys of Agriculture and Agrifood Canada.

"You can't make potato bread and you really can't make rice bread and that's what makes wheat so special. You have these proteins in wheat which allow you to make a staple for billions of people around the world and when you're feeding that many people it becomes very important."

If there's any doubt about the importance of wheat consider this. The modern Canadian wheat crop generates between $3 billion to $5 billion worth of sales world wide, depending on that year's production and prices. Add to that the secondary industries, the milling and baking, and you have an additional $7 billion or $8 billion dollars of value-added.

The industrial might of common bread wheat, is founded on a combination of two proteins glutenin and gliadin which, when bolted together, form the elastic protein gluten. It's gluten that makes the loaf of yeast bread light and fluffy and puts the crumbly texture into that chocolate cake.

"Gluten gives this elastic property to the dough." says Swift Current wheat breeder Ron Depauw. "When you mix the starch and protein together with water and yeast the starch gets converted to a sugar. When the yeast digests the sugars it gives off carbon dioxide which gets trapped within this protein/starch matrix."

If you've ever made bread you know exactly what Depauw is describing. As you knead the dough it takes on a stretch consistency with little bubbles that form, and you can feel them popping as you press down the dough.

As the yeast belches carbon dioxide, the bubbles are trapped in the protein causing the dough ball to rise. When you take a new loaf out of the oven and carve off a slice, as the fresh steam wafts from the newly minted bread you can see the remains of those gas bubbles. The result is a light, chewy slice and maintaining this quality is the first thing a wheat breeder has to consider. It's equally important for other products like cakes and cookies, which rely on the chemical reaction of baking powder to produce carbon dioxide.

This was a problem with Garnet wheat, released in 1926. It's agronomic properties were very good and it matured earlier than Marquis so it was popular with producers. Not with customers, however. It simply didn't make a good loaf of bread and that spelled its demise.

Still, if the farmer can't grow wheat then Canada can't sell wheat — but at the same time, if processors don't like the varieties Canadian farmers are growing, they can buy similar quality wheat from the U.S. or Australia, to name just two of our top competitors.

The different types of baked goods are also changing with the times so it's not all about white bread anymore. There's a whole variety of products like bagels, pizza dough, flat breads and frozen doughs that may require some tinkering with the genome.

The UK bakers Warburtons, for example, like Canadian wheat so much they've begun pushing the wheat's quality as part of what makes their bread so good, and they've been contracting acreage to ensure their needs are met, restricting participation to a handful of wheat varieties.

Along with the standard bread wheat there are also other important wheat types — durum for pasta, or the new general purpose wheat classes aimed at providing high-yielding varieties for ethanol and animal feed.

As end use customers begin to specify more and more functional characteristics for the varieties they choose to buy and more uses for wheat develop, there's little doubt that the only people who will be able to address these emerging issues are Canada's wheat breeders.

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complex genetics of wheat makes this an even larger headache for wheat breeders, as the three separate genomes fight each other for dominance in wheat.

It's the genetic equivalent of pulling the handles of three slot machines lined up in a row at the same time — and hoping you hit the same winning sequence on all three. The odds are huge and the mathematical probabilities alone are mind boggling.

When these complex combination of individual genes line up to create a wheat line, what you've then got is a genotype. And then Mother Nature goes to work on said genotype, mercilessly applying selection pressure to the resulting wheat line.

This isn't news to any farmer who's ever watched a crop emerge and then be attacked by insects or disease. Some genotypes fare better than others and survive while others are easy prey and quickly overcome.

This tug-of-war between plants and pests has been going on for millions of years and Mother Nature handles this in two different ways.

First, natural plant communities are very diverse, with a lot of different plants jostling for real estate. Unlike farmers, Mother Nature isn't interested in growing fields of genetically identical plants that grow, thrive and yield a profitable return.

She's more interested in filling vacuums and when one particular plant gets wiped out, others can't wait to fill in the space that's opened up — which is why you'll never see anything even remotely like a farm field growing naturally.

Then there's the genes of the plants themselves. When pressure is applied over time, the population will eventually develop natural resistance or perish. When resistance does develop, the plants will thrive — but then the pest and disease itself becomes threatened, meaning it begins to develop genetically to overcome the resulting plant resistance.

It's this process that naturalist Charles Darwin described as natural selection which is often characterized as a tooth-and-nail struggle for survival, but in reality it's a more passive process. Winners generate more offspring than the losers and as a result Mother Nature keeps populations small and plant communities diverse and stable.

To a plant breeder, this means that if a stand of plants is attacked by a disease like fusarium, the genetic combination that survives will be the one that is carried on to the next generation. The strong survive, the weak are weeded out and ultimately the overall population will adjust and adapt. When a plant breeder goes to work, what they're really trying to do is hurry this process along by speeding up the selection process, sort of like a hurry-up offence in football.

"You've got to know where you're going to go and what traits your trying to bring together in order to get there," DePauw says. "Once you know what traits you need, you look for the ones with the complementary characteristics that make it theoretically possible to find the unique individual that would have all of the traits of your objective."

It also means that wheat breeders don't just need to understand the extremely complex plant they're working on. They also need to know how the pathogens that are attacking it work.

If you're going to breed resistance you have to know where the enemy is weak so the wheat can parry its attack. Stephen Fox of Agriculture Canada in Winnipeg is breeding midge resistance into wheat and the input he gets from entomologists and biochemists is absolutely indispensable.

"You have to understand the biology of the system you're dealing with," he says. "Some kind of disease or insect resistance is different than dealing with a seed quality characteristic. Understanding its biology helps you figure out how to structure your breeding program."

Since plants learn to cope by adjusting their genetics to deal with the pathogen in question, the trick is to find which genes drive the plant's defence mechanisms and find out how to manipulate them to the farmer's advantage — but the results can at times be clearer than the reasons why.

"We've found that the adult midges don't want to lay eggs on certain lines for some reason," Fox says. "When they do eventually get around to laying eggs larvae do fine but they don't lay as many eggs there."

There seem to be a couple of ways that Fox and other breeders have short-circuited the midge's relationship with wheat. The female midges are tiny and they like the humid environment under the canopy. They lay their eggs on the kernels during warm, calm nights as the wheat is flowering. The young hatch and spend a few of weeks feeding on the kernels before they drop to the soil and pupate. In one case they've found that certain lines produce a plant acid called phenolic acid which the midge larva don't like.

"Even the susceptible wheats produce these things but they start producing them later," Fox said. "The ones that carry the gene produces phenolic acid a lot sooner. When the larvae emerge they don't like what's available so they starve to death."

Another line tricks the female into laying her eggs on the rachis, the part of the stem where the seeds are attached, so when they hatch the tiny larvae have to undertake a long journey to the seeds. It's too long a journey so they starve. Both strategies depend on the breeder's understanding of how the midge works.

So you've drawn the road map, you've selected the parental material, you know how the pest works and now you're ready to make the first cross. Pollen from one line is applied to the flowers of another. In this way, the arms of all those slot machines are pulled, the wheels turn, the billions of different combinations click into place and the first round of seeds are produced.

This generation of seeds is called the F1 or first filial generation. Somewhere in there you hope to have the perfect combination of genes that will give rise to the perfect plant where all the dominance issues between genes, alleles and genotypes have been settled perfectly in your favour. This perfect plant is called the homozygous plant, which means it has all the right alleles for a single trait, such as midge resistance — but finding that perfect plant is like a needle in a really big haystack.

"In the simplest case where the two parents differ by only one gene in all 21 chromosomes the number of different combinations you can get is 2 to the 21st power which is over two million," Depauw said. "This starts getting a bit complicated. There's many many genes on any one of those chromosomes and even if one plant actually has all of them in a homozygous condition, your chances of finding it are almost zip."

However, a number of the offspring will be close enough to be contenders and the way to find out is to push the selection the way Mother Nature does. We subject them to the same pressures, so they're deliberately infected with the target disease or set up to be attacked by the chosen insect.

Survivors in these trials go on to breed the F2, F3 and F4 generations and so on. After several generations of these crosses the end of the roadmap comes in sight as the traits generated by the breeding program start to show up consistently. The plants are now breeding true.

Only then is the new line is ready for field trials where the agronomics and productivity are compared to an established breed, usually AC Barrie. Once it's passed all of the tests it's recommended for registration and ready for the farmer's field and the dinner tables of the world. The elapsed time? About eight to 15 years, if everything goes just right.

Since it takes that much time, not only do wheat breeders have to solve our immediate problems as they did with fusarium and wheat midge. They also have to try to look ahead to see what pests the future may bring.

"Certainly we're always looking to improve yields and we're looking to incorporate better disease resistance." says Agriculture Canada's Robert Graf in Lethbridge. "There are these new races of stem rust in Africa that defeat many of the effective genes that we have in our current wheat varieties. When these races come to North America much of our stem rust resistance will no longer be effective."

While the issue of wheat genetics is clearly a complicated one, there is one thing that is clear — as long as we continue to grow wheat we're going to keep manipulating it's huge and complex genome.

New advances on computer power and breeding techniques should make that even better and may help to shorten the time taken to get effective new lines on the market. In the end, however, it all still rests on the shoulders of those who unravel and manipulate crop genomes. ■