SCIENCE TO HELP WHEAT SLEEP THROUGH PRE-HARVEST RAIN

Preventing costly pre-harvest sprouting is within reach of plant breeders following a breakthrough CSIRO gene discovery technique that has found two genes related to an important evolutionary protective trait – grain dormancy

By **Dr Gio Braidotti**

■ With the need to meet stringent quality requirements, wheat varieties that succumb to pre-harvest sprouting (PHS) are a costly frustration for growers, typically lowering grain quality from premium to feed grades as well as reducing yield.

A survey of the 2004-05 season in Western Australia, for example, revealed growers lost 22 per cent of their grain's value due to PHS-related downgrading.

The economic cost for an individual grower can be in the range of $50,000 to $100,000 in the years PHS occurs. In 2010, losses in South Australia, New South Wales and Queensland were said to total as much as $100 million.

Breeders know that the most effective way to reduce PHS is to select varieties with increased seed dormancy – in other words, a more stable germination mechanism that minimises the chance of wheat grain responding to pre-harvest rain.

For ancestral wild wheats, the progenitors of modern bread and pasta wheats, grain dormancy was a survival mechanism. It prevented germination until the conditions were right for seedling growth when cooler, more steady rain periods arrived in autumn to spring. However, during the long process of domestication this dormancy trait was one of the many genetic mechanisms suppressed as organised agriculture developed. Today, it is the absence of this protective mechanism that can induce PHS in the event of late-season rain.

Restoring this trait has been a quest for some time, but it is a complex trait of expression that is affected by environment, making genetic screening difficult. Also dormancy is undermined by selection for rapid and uniform germination that maximises modern-day farming's need for synchronised plant development.

The hoped-for solution to this catch-22 situation has been to one day isolate dormancy genes for independent inclusion in marker-assisted breeding programs.

That day has arrived.

Dr Frank Gubler (left) and Dr Jose Barrero at a field trial during the hunt for wheat genes that suppress pre-harvest sprouting.

PHOTO: CSIRO

Two dormancy-related genes (*PM19-A1* and *PM19-A2*) have been isolated by Dr Frank Gubler's CSIRO team in a GRDC project that forms the primary research focus of his colleague Dr Jose Barrero.

"We think that both genes could be used to regulate grain dormancy in breeding programs," Dr Gubler says.

The team used a novel combination of technologies – some developed exclusively by CSIRO – and were assisted by a team of bioinformaticians and statisticians from CSIRO (Dr Klara Verbyla, Professor Arunas Verbyla, Dr Emma Huang, Dr Stuart Stephen, Dr Penghao Wang and Dr Alex Whan) and the Victorian Department of Economic Development, Jobs, Transport and Resources (DEDJTR) (Dr Matthew Hayden and Dr Josquin Tibbits). Previous knowledge accrued by Dr Daryl Mares and his team at the University of Adelaide was also drawn upon.

The identified genes are located on the long arm of chromosome 4 (from bread wheat's A genome) and can contribute up to 40 per cent of the dormancy variability detected in some wheat populations. The genes were possible to detect because it has been found that incongruities in a trait within a population give away the location of the gene or genes responsible. The dormancy gene has been found to be an example of this in that it is associated with both dormancy and its opposite effect, pre-harvest sprouting. At high levels of expression it is associated with dormancy and at low levels with pre-harvest sprouting.

Such incongruities allow a gene to stand out from the background genetic noise and this is the basis of gene mapping.

To better exploit the gene-revealing power of such biodiversity, Dr Colin Cavanagh (previously with CSIRO and now with Bayer CropScience) developed several gene-mapping populations that maximise genetic diversity within the smallest possible collection of wheat lines.

These are called the Multi-parent Advanced Generation Inter-Cross (MAGIC) populations. They can be used to isolate genes and markers for any trait of interest to pre-breeders.

The MAGIC population used to locate the dormancy genes is a 'four-way' population of 1100 lines. It was developed by crossing four Australian wheat varieties to sample the genetic diversity and heritage of Yitpi®, Chara®, Baxter® and Westonia.

An eight-way MAGIC population also exists that samples diversity from all over the world and it is currently being used by Dr Barrero to search for additional dormancy genes to build even more robust resistance to PHS.

HOW TO FIND A WHEAT GENE

CSIRO's strategy started with field trials of the four-way MAGIC population, testing for dormancy and pre-harvest sprouting.

A high-density DNA chip provided a bridge between observed differences and the underlying genetic incongruities.

This DNA chip contains 90,000 markers that can distinguish tiny differences in the DNA inherited from the population's four parents. This provides a framework for understanding which bits of DNA are co-inherited with dormancy.

Dr Barrero's scientific breakthrough was to increase the resolution of gene-mapping techniques to reveal the presence of the two dormancy-related genes *PM19-A1* and *PM19-A2*.

"The results clearly pointed to a gene whose expression is elevated in dormant lines," Dr Barrero says.

The analysis of this expression data was made possible by the expertise of CSIRO and Victorian DEDJTR in genomic analysis and also to the availability of the wheat genome draft from the International Wheat Genome Sequencing Consortium.

"We found it was the Yipti® parent in the four-way MAGIC population that confers a higher expression of the dormancy gene *PM19-A1*," Dr Barrero says. "But we have now detected this dormancy gene in other varieties, such as Soleil, Cayuga, Halberd, AC Barrie."

This means the crucial dormancy gene has been found in the Australian gene pool and that markers also exist to now select this trait for future crop improvement.

Delivery to breeders

The trait breeder at the LongReach Plant Breeder, Dr Marie Appelbee, sees the discovery of the dormancy genes as important wherever there is a chance of rainfall post-ripening but pre-harvest.

"Currently, that's a significant issue, partly as a consequence of breeders' own actions. Given today's fast breeding regimes we can inadvertently reduce dormancy," she says.

"It is a catch-22 but markers cut through that, especially if included early in a breeding pipeline so that we can cull susceptible material."

Dr Appelbee explains that trait breeders like her are the contact point in breeding companies for the use of pre-breeding technologies and discoveries, such as the *PM19-A1* and *PM19-A2* genes.

Asked about uptake of pre-breeding trait discoveries more generally – and what influences a commercial breeders' decision to adopt novel traits – Dr Appelbee says it is a question she is frequently asked as growers may not be seeing the traits they read about because it takes eight to 10 years to complete a breeding cycle and release a new variety.

"My position is that when pre-breeders provide germplasm containing the trait, and a marker to select for it, I will make crosses to that germplasm and use the marker to select and retain the trait of interest," she says.

"For me, markers are the most important tool for incorporating a new trait. We simply can't afford the cost and the time to undertake complex testing in field trials."

Besides dormancy, several pre-breeding discoveries have excited Dr Appelbee's interest. These include:

- resistance genes to yellow leaf spot, crown rot, rusts and nematodes;
- alternative dwarfing (or Rht) genes (*Ground Cover Supplement* March–April 2013) that deliver longer coleoptiles developed by CSIRO's Dr Greg Rebetzke in partnership with a private company;
- the salt-tolerance *Nax-1* and *Nax-2* genes (*Ground Cover* May–June 2012) that are making their way through the LongReach development pipeline and are in quite advanced yield trials;
- the trait associated with both heat and frost tolerance during flowering developed by CSIRO's Dr Rudy Dolferus (*Ground Cover* March–April 2014); and
- Dr Daryl Mares' efforts to map and develop markers for late-maturity alpha-amylase (*Ground Cover* November–December 2014).

Plant breeder Dr Marie Appelbee.

PHOTO: GIO BRAIDOTTI

On both a scientific and historic note, it was the DNA sequence data for *PM19-A1* that then revealed a second gene (*PM19-A2*) adjacent to the first.

Gene-silencing experiments using CSIRO's RNAi technology revealed that both genes are needed by Yitpi[Φ] to activate its dormancy protection against PHS.

In terms of knowing which grain-growing regions these genes will work in, given not all varieties are suited across the country, Dr Gubler says it is not known how these genes actually confer dormancy, although the researchers do know the expression of the genes is suppressed by high temperatures during grain development – and this of course then elevates the risk of PHS.

This means these genes slightly favour southern and western-region varieties.

"The future challenge is to map dormancy genes that are resistant to high temperature – or to exploit variants of the *PM19-A1* and *PM19-A2* genes that have lost their responsiveness to heat. Then we can build dormancy protection that will work well in both cooler and warmer environments," Dr Gubler says. □

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Pre-harvest sprouting weather damage. The discovery of wheat's long-lost dormancy genes may prevent future varieties from reacting to late-season rain.

Genetic variation within the bread wheat gene pool exists to better prevent yield and quality losses from pre-harvest sprouting.

FERTILISER DATA GAP HAMPERS PLANNING

By Catherine Norwood

■ A lack of national fertiliser-use data is hampering crop predictions and has the potential to cost growers millions of dollars in lost income when the grains handling system is 'caught out', says grain supply dynamics specialist Dr David Stephens.

Dr Stephens says there has been no regional-scale fertiliser-use pattern established on a national level for 15 years – the last time this information was included was in an Australian Bureau of Statistics agricultural census.

He says the level of nutrients used, particularly nitrogen, can be a key guide to yield and crop forecasts, and the absence of this information leads to poor planning. He points to the 2010 harvest in southern Australia that was much larger than expected, causing extensive and costly bottlenecks in farm-to-port delivery.

"The volumes caught growers and grain handlers by surprise. There were long queues at receival depots, and as trucks were waiting in line the weather turned. Heavy rain resulted in a significant downgrading of the remaining crops; it cost growers millions of dollars."

Dr Stephens, who works with the Australian Export Grains Innovation Centre, says nitrogen fertiliser is the second most important driver of crop production across Australia behind rainfall: "But the lack of nutrient data, once determined by an annual census, severely affects national and shire crop yield forecasts."

Dr Stephens sees it as a significant gap in the available market intelligence that, if addressed, could improve growers' operational decisions, as well as the provision of grain transport and storage services and grain trading decisions.

"Australian cropping systems have the highest yield variability of anywhere in the world and the need for more accurate production predictions that benefit both growers and markets is greater than ever," Dr Stephens says. □