

# Hunting for genes

FALLING NUMBER PROJECT SEEKS TO REDUCE RISK BY BREEDING FOR GENETIC RESISTANCE

By Camille Steber, Michael Pumphrey and Arron Carter

In 2016, Eastern Washington farmers were surprised when a record-yielding harvest was marred by steep discounts caused by low falling numbers.

The Hagberg-Perten Falling Number (FN) Test measures starch degradation by the alpha-amylase enzyme based on the time in seconds needed for a stirrer to fall through a gravy made by heating and stirring a flour/water mixture. Alpha-amylase chops up long starch chains into shorter starch ones, lowering the gelling capacity of the gravy and the time required for the stirrer to fall through the gravy. Most foreign customers tender for a minimum falling number of 300 seconds because poor end-use quality, including falling cakes, sticky bread and mushy noodles, are associated with lower scores.

As luck would have it, the 2017 crop showed little or no falling number problems. Based on a survey of 672 Washington State University (WSU) Cereal Variety Trial entries, only seven soft white winter and four soft white spring locations had few varieties under the 300 second mark (<http://steberlab.org/project7599.php>). Compare this to 17 soft white winter and 14 soft white spring locations that had many low FN values in 2016.

As in 2015, we found many of the 2017 samples with FN under 300 seconds did not have elevated alpha-amylase levels. Elevated temperatures during grain filling in the spring may have caused poor starch accumulation in the grain, but this caused only a mild reduction in FN. Relying on luck to prevent discounts due to low FN, however, is not an option. The goal of the Washington Grain Commission-funded Falling Numbers Project led by Camille Steber, Michael Pumphrey and Arron Carter, is to reduce risk by breeding for genetic resistance to low FN.

Breeding for higher FN is complicated by the fact that alpha-amylase can be triggered by two causes: preharvest sprouting and late maturity alpha-amylase. Preharvest sprouting is the germination of grain on the mother plant when rainy conditions occur before harvest. Late maturity alpha-amylase (LMA) is the induction of alpha-amylase by large temperature swings during late grain filling.

Problems with low FN in 2013 were due mainly to sprouting, and to a lesser extent, LMA. Preharvest sprouting was prevalent in both soft white winter and spring wheat. Thus, the initial emphasis of the project was to map genes for preharvest sprouting tolerance. Resistance to preharvest sprouting largely results from high grain dormancy at maturity.

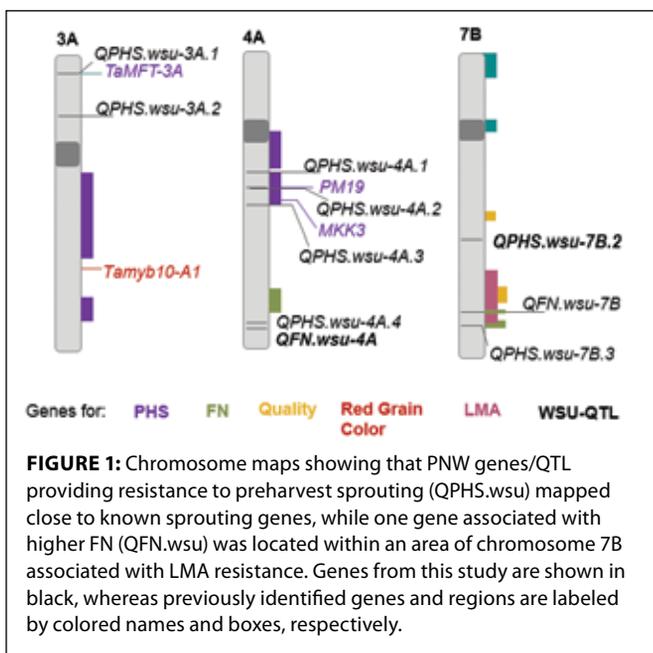
Dormant grain cannot germinate, even if it gets wet. Dormancy is lost either through a period of dry storage called after-ripening or by the combination of rain with cool temperatures. Dormancy loss is the reason that wheat is more likely to sprout if the temperatures drop during a rain event. After-ripening is the reason that sprouting is more likely if the wheat has stood dry in the field for awhile after maturity.



The Steber lab studies resistance to low falling numbers. From left to right, front to back are Camille Steber, Stephanie Sjoberg, Courtney Broedlow, Wenjing Ge, Rehana Parveen, Tracy Harris, Samantha Conselman, and Shantel Martinez.

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Shantel Martinez (left) and Rehana Parveen take notes on a winter LMA mapping population. Martinez mapped genes for preharvest sprouting and low FN tolerance. Parveen led the LMA effort in the 2017 field season.

Preharvest sprouting tolerance genes were mapped in a four-year, genome-wide association study using a panel of 470 soft white winter wheat lines representing seven northwest breeding programs. Quantitative trait loci (QTL) were mapped based on falling numbers in the field after natural or artificial rain (called QFN.wsu) and on controlled greenhouse preharvest sprouting using spike-wetting tests (called QPHS.wsu). We use the term QTL instead of gene because at this point, we can't judge if we're dealing with one or more genes at a given map location.

Nine QFN.wsu associated with higher FN and 34 QPHS.wsu loci associated with sprouting tolerance were identified. Two QPHS.wsu mapped close enough to cloned sprouting tolerance genes, MKK3 and MFT-3A, that we think they may be the same genes (see Figure 1). Interestingly, the QFN.wsu QTL mapped using grain grown in Central Ferry, Wash., in 2016 landed in a region of chromosome 7B containing a large QTL associated with LMA resistance, as well as known quality and FN QTL. This points to the importance of LMA in determining 2016 falling numbers.

While the first mapping study provided the molecular markers needed to select early breeding lines for preharvest sprout resistance, ongoing work will balance this with good seedling emergence. If grain remains dormant too long after maturity, then it may cause problems with

poor germination and emergence, especially from deep planting. A combination of genes that gives good dormancy/sprouting tolerance at maturity that is lost within 6 to 10 weeks of after-ripening is needed to avoid problems with poor emergence in winter wheat. This is being done by comparing sprouting scores and FN to emergence data for two mapping populations. A genomic selection strategy will be developed in cooperation with WSU genomic statistician, Zhiwu Zhang.

The 2016 field season was a big eye-opener, because there was a widespread problem with LMA compounded by some preharvest sprouting. Whereas red kernel color provides some resistance to sprouting, it does not provide resistance to LMA. So we wound up with low FN problems in both soft white and hard red cultivars.

In the 2017 field season, we launched an intensive effort to map LMA loci in winter and spring wheat. To do this, we had to greatly scale up field LMA testing. Wheat spikes were harvested between 26 and 30 days after pollen shedding and subjected to a cold shock for seven days. Thus far, 160 of 1,200 lines have been checked for LMA using FN and alpha-amylase enzyme assays.

The data looks good because low FN values are associated with elevated alpha-amylase levels. The goal is to map LMA resistance genes in Northwest wheat, thereby improving our ability to breed for stable falling numbers, an outcome based on science, not luck. ■

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