

Late-Maturity α -Amylase—Apparent Sprout Damage Without Sprouting

Consider this scenario: a breeder has been thrilled with the performance of a new variety. It yields well, its pathogen resistance is standing up well, and millers have liked the early samples provided to them. Then suddenly during the harvest, when the variety is really on trial, harvested loads from some growth regions are rejected due to low falling number values (200 sec or so) when other varieties in the same region are completely sound. The low falling number values are verified by laboratory testing and found to be the result of elevated levels of α -amylase activity. This defect cannot readily be explained because there has been no rain at harvest and no grains show visible signs of sprouting! What has gone wrong?



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Late-Maturity α -Amylase—A Lurking Defect

There is a good chance that the elevated levels of α -amylase activity are due to the presence of genes for late-maturity α -amylase (LMA), also termed “pre-maturity α -amylase” (3, 4,8,10,11). This genetic defect has appeared in the progeny of many recent crosses and is present in many breeding programs (a prevalence of up

to 20% has been estimated). Many breeders are now aware of the difficulty posed by this defect, and efforts are underway to eliminate it from breeding programs. Its presence is difficult to detect, however, because the expression of elevated α -amylase levels is only evident under certain growth conditions. The scenario described above, thus, is quite possible, i.e., the defect may not be identified until after a variety has been released commercially.

The result of the LMA defect is similar to preharvest sprouting in mature grains in which starch-degrading enzymes are active, but grain growers are likely to be unaware of the problem because there are no external signs of the defect—no splitting of the germ case and no rootlets appearing. LMA is “invisible” until some form of enzymic testing is performed on the delivered grain, such as falling number testing or other methods that detect sprout-related activity. It may even remain undetected until the grain is processed through the mill and bakery.

Unlike sprout damage, LMA may be triggered by low temperatures (probably a cool-temperature shock) during the second half of the grain-filling period, e.g., 12–18°C at 25–35 days after flowering (8). By contrast, conventional sprout damage is triggered by rain falling on the ripe grain. Furthermore, the distribution of enzymic activity in the endosperm is distinctly different in the two cases. Although α -amylase is secreted by the aleurone layer in both LMA and sprouting, the distribution of activity for grains with LMA is approximately even from germ to brush end, whereas the enzyme is concentrated near the embryo in germinating grain (11).

Detection of the LMA Genotype

Both conditions have significant genotype–environment interactions, i.e., some varieties are more susceptible to developing LMA or sprouting than others following an environmental trigger. In the case of sprout damage, the genetic factors appear to be complex. Quantitative trait loci (QTL) have been identified on chromosomes 2AL, 2DL, and 4AL (5,7) and also on chromosome 1AS and Group-2 chromosomes (2). Recent evidence suggests that the 4A QTL is important in genotypes of diverse origin (6). Furthermore, genes for dormancy (tolerance to sprouting) have been reported for chromosome 3D and are associated with the genes for red-grain color on Group-3 chromosomes.

The genetic influences for LMA appear to be simpler, possibly involving only two independent genes with QTL on chromosome arms 3BL and 7BL (5,9,10). These QTL are related to a range of genotypes, but an even wider range of genotypes still must be screened. The presence of either one of the genes is sufficient to produce the LMA defect; the presence of both is more serious. The effects of these QTL may be less in the presence of semidwarfing genes (*Rht1* and *Rht2*) and greater for 1B/1R varieties.

LMA is difficult to detect because it appears only under certain environmental conditions. To aid in detection, some breeders now include at least two check varieties in their plots: one variety known to be an LMA genotype and the other known to be free of this genetic defect. Preferably, the check varieties should have similar maturity. The harvested grain is tested for amylase activity using Falling Number equipment, a test kit (e.g., WheatRite), or another method of determining α -amylase activity. If the LMA genotype variety has enzymic activity but the LMA-free variety has no amylase activity, it is likely that the growth conditions suited the appearance of the defect and, thus, the remaining progeny should be tested for its presence. Alternatively, LMA expression can be induced by subjecting whole plants or detached tillers to a cool-temperature shock midway through grain filling (8).

The more direct approach is to attempt to develop a screening method to detect the presence of the requisite gene(s) at the DNA level. This has also proved to be a possible tool in breeding, but not yet as a method for analyzing grain deliveries. Microsatellite markers that flank the 7B locus or are linked to the 3B locus are now available for marker-assisted selection in breeding.

α -Amylase Isoenzymes

The type of enzyme involved in LMA and sprout damage appears to be the same. During the early phase of grain filling, there is normally modest α -amylase activity produced by low-isoelectric point (pI) isoenzymes that are present in the pericarp tissue under the control of the α -Amy-2 genes located on Group-7 chromosomes. A residue of these isoenzymes may account for elevated α -amylase activity in frosted grains. Normally, α -amylase activity declines during the ripening process, falling to a negligible level by harvest (3).

The normal germination process, triggered by moisture after a period of dormancy, initially involves the production, first by the scutellum and then by the aleurone, of a distinct group of α -amylase isoenzymes with high pIs that are products of the α -Amy-1 gene family located on Group-6 chromosomes. Later during germination,

the low-pI group is also synthesized (by the aleurone rather than the pericarp), although this is generally beyond the point at which the level of α -amylase activity is unacceptable to industry. The α -Amy-1 gene family produces the enzymic activity associated with both LMA and the early (industrially significant) phase of the germination process and, thus, also with sprout damage. This similarity in isoenzyme type means that antibody-based kits for rapid determination of sprout damage (12) can also be used to detect the presence of LMA, even in the presence of variable amounts of residual low-pI α -amylase isoenzymes, offering a simpler approach to its detection.

Processing Consequences of LMA-Affected Grain

Although grain is liable to be downgraded if it has a low falling number value (whether due to sprouting or LMA), results suggest that the effects of LMA on baking quality are not as serious as might be expected if the same falling number value were due to sprouting (1). Falling number values for 23 samples of the Australian variety Chara ranged from 150 to 400 sec; the low values were due to LMA, not sprouting. Quality testing of the resulting flour samples showed no significant correlations between falling number and loaf volume (test baking) or noodle-sheet color (after 0.5 and 24 hr), whereas both of these quality attributes are related to falling number in sprout-affected samples in this range. The reason for this apparent anomaly may lie in the fact that LMA-affected samples do not have the range of additional enzymes (e.g., proteases) that are activated by the germination process.

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