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Review

Late-maturity α-amylase: Low falling number in wheat in the absence of preharvest sprouting

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Abstract

Late maturity α-amylase (LMA), or prematurity α-amylase (PMAA) as it has been termed in the UK, in wheat involves the untimely synthesis of high pI α-amylase during the middle to later stages of grain development and ripening. The enzyme activity is retained in the grain at harvest ripeness, resulting in low falling number and failure to meet receival standards and customer specifications. This phenomenon, which is restricted to specific genotypes, appears to be controlled by 1 or 2 recessive genes acting alone or in combination and in most cases appears to be triggered by a temperature shock. This shock is only effective if it occurs during a window of sensitivity around 25–30 days postanthesis. Expression of LMA is reduced in the presence of dwarfing genes such as *Rht1*, *Rht2* and *Rht3* that confer insensitivity to gibberellin. Screening technologies, including molecular markers and high pI-specific ELISA, have been developed to assist wheat breeders and will be required to meet new challenges posed by novel germplasm such as primary synthetic wheats.

Keywords: Wheat; Late maturity α-amylase; Falling number

Contents

1. Introduction . . . 7
2. Falling number and measurement of α-amylase activity . . . 7
3. Incidence of late maturity α-amylase . . . 8
4. α-Amylase in developing, germinating and LMA-affected grains . . . 9
5. Effect of LMA on end-use and end-product quality . . . 10
6. Variation in LMA expression . . . 10
7. Interaction with the environment . . . 10
8. Interaction with dwarfing genes . . . 12
9. Genetic control of LMA including QTL and markers . . . 12
10. Possible mechanisms involved in expression of LMA . . . 13
11. Determination of LMA phenotype . . . 14
12. New challenges . . . 15
Acknowledgements . . . 15
References . . . 15

Abbreviations: ABA, abscisic acid; dpa, days postanthesis; ELISA, enzyme-linked immuno sorbent assay; GA, gibberellic acid; gamyb, GA regulated transcription factor; LMA, late maturity α-amylase; pI, isolelectric point; PMAA, prematurity α-amylase; QTL, quantitative trait locus

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1. Introduction

Hagberg (1960, 1961) and Perten (1964) developed the falling number method as a simple and rapid technique for determining α-amylase activity using wheat meal as the native substrate. Subsequently, this method has become the international standard (AACC (American Association of Cereal Chemists), 1972; ICC (International Association of Cereal Science and Technology), 1968) that is used widely in grain classification, quality control and marketing. Grain with a low falling number due to high α-amylase activity causes substantial economic losses to growers, significant processing and storage problems and is generally reflected in poorer quality end-products (Derera, 1989; Edwards et al., 1989). Indeed with the advent of highly automated food production plants, particularly bakeries, variation in α-amylase in the starting material is now even more undesirable. Low falling number is generally associated with preharvest sprouting; however, it is now clear that there are a number of additional causes of low falling number. These include late maturity α-amylase (LMA) (Mares and Mrva, 1993) also known as prematurity α-amylase in UK (Gale et al., 1987) and retained pericarp α-amylase (Kettlewell et al., 1996).

LMA presents a significant challenge both to scientists, since it appears to be a genetic defect in the sense that it is limited to particular genotypes that are subject to rather complex modulation by environmental factors, and to the wheat industry where the absence of physical signs of damage and an almost non-existent capacity to predict its occurrence presents a substantial classification and quality management dilemma. This review will focus primarily on a discussion of LMA whilst drawing some parallels with preharvest sprouting or germination.

2. Falling number and measurement of α-amylase activity

The falling number method is a viscometric assay that involves the rapid gelatinization of a flour or meal suspension in water, by immersion in a boiling water bath, with subsequent measurement of the liquifaction of the starch by α-amylase. In modern, semi-automated instruments, a motor commences the stirring action after 5 s and continues for a further 55 s. At the end of the mixing cycle, the viscometer stirrer or spindle is released from its top position, falls through the gelatinized suspension under the influence of gravity and the time required to fall a set distance is recorded in seconds which represents the falling number. Increasing levels of α-amylase result in a decrease in falling number down to 60 s, beyond which further increases in activity cannot be measured. Because α-amylase is an endo-acting enzyme, that inserts breaks in the interior of the very large starch molecules, small amounts of enzyme cause dramatic reductions in viscosity. This is reflected in an inverse curvilinear relationship between α-amylase activity and FN (Barnes and Blakeney, 1974; Mares, 1987). Wheat grains have the capacity to synthesize very large amounts of α-amylase. However, the activity required to reduce FN below receival limits is very small, only 2–3 times the levels found in sound grains (Mares, 1987). Many countries use the falling number method at grain receival and as a component of trading specifications. FN values above 250, 300 s or in some cases 350 s are required for inclusion of delivered grain in high-quality grades depending on the receival standards set by the wheat industries in different countries.

In the falling number test, the time required for the flour/water suspension to pass from the starch gelatinization temperature when starch becomes very accessible to attack by α-amylase to enzyme inactivation is only of the order of 30 s and takes place during the initial 60 s of the test. These conditions are far removed from most industrial processes such as baking. Nevertheless, the test has a number of important advantages including speed, reproducibility, simplicity and cost that make it very useful as an objective test for purchase or grading purposes.

The FN method measures differences in both enzyme and substrate, and the results are influenced by genotype and environmental conditions under which the grain samples developed and matured. An extreme example of genotypic variation in substrate involves the reduction in the amylose content of starch in waxy wheats (Graybosch et al., 2000) that have substantially lower FN compared with controls. A measure of the inherent viscosity of the substrate, in the absence of α-amylase, can be obtained by using a brief acidification treatment (Meredith, 1970) or addition of silver nitrate (Batey et al., 1997, 2001) or mercuric chloride to inactivate the enzyme.

Whilst falling number is used universally at grain receival to assess grain quality and α-amylase activity, the Amylograph and the Rapid Visco Analyser (RVA) are the instruments more commonly used by the milling, baking and grain export industries. As with falling number, these instruments measure differences in both α-amylase activity and substrate; however, whilst both are very time consuming they provide additional important information about starch properties that are relevant to processing (Batey et al., 2001; Mares, 1989).

A wide array of analytical methods are available for the specific measurement of α-amylase activity (Kruger, 1989). In general, these require extraction of the enzyme from the sample matrix and activity is then measured by the rate of hydrolysis of dye-labelled starch or other model substrates under controlled conditions. Specific isozymes of grain α-amylase can be separated and visualized by isoelectric focusing (Gale and Ainsworth, 1984; Mares and Gale, 1990) or quantified using isoelectric focusing in a liquid column (Mrva and Mares, 1999). Antibodies specific for α-amylases in developing or germinated grains were described by Daussant and Renard (1987) and used to determine the tissue location and ontogeny of amylases in developing wheat grains. More recently, polyclonal and monoclonal antibodies that are specific for the high pI