Late maturity α-amylase (LMA) in wheat

Summary
Late maturity α-amylase (LMA) is a grain defect found in wheat germplasm throughout the world that can result in unacceptably low Falling Number. The defect is genetically controlled but expression is strongly influenced by the environment. Effective screening technologies, including molecular markers for several genetic loci associated with the trait, have been developed and are currently used to support wheat variety classification as well as phenotyping for ongoing research. Significant advances have been made in understanding the mechanisms involved and the complex interaction with environment. In addition, there is strong evidence for a genetic locus that suppresses LMA expression.

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Conclusions
LMA remains a significant constraint for Australian wheat breeding companies and they discard significant numbers of breeding lines on the basis of LMA level very late in the variety development process. This limits the opportunity for genetic gains in yield and other important traits. Despite the improved screening protocols based on phenotype and use of the lower benchmark, Wyalkatchem\(^5\), for classification, the failure rate due to LMA is still unacceptably high (>30%). In most, if not all, of the sources of LMA that have been investigated, the major quantitative trait loci (QTL) associated with genetic variation was located on chromosome 7B.

Single nucleotide polymorphism (SNP) based Kompetitive Allele Specific PCR (KASP) markers identified in this project that are suited to both high throughput marker technologies and early generation selection should be a welcome acquisition for breeders. While this represents a significant advance, it is important to note that: these markers are linked to, but not diagnostic for, the genes underlying LMA QTL on 7B, 3A, 3B and 2D; effective deployment of these markers depends on breeders knowing the sources of their LMA; LMA can reappear in cross populations if apparent non-LMA parents either carry different but complementary LMA QTL other than 7B or one of the parents, e.g. Hartog, carries LMA 7B in combination with a ‘suppressor’ locus located elsewhere on the genome. The ‘suppressor’ will be the subject of intense investigation over the next few years. The current phenotyping protocol for identifying LMA in semi-dwarf backgrounds depends on delivering a cool temperature shock (12°C night/18°C day for seven days) commencing approx. 25 days after anthesis to plants, or detached tillers, which have been grown at daily temperature maxima greater than 25°C post anthesis. In this project, constitutive expression of LMA in semi-dwarf backgrounds has been demonstrated under controlled environment conditions based around limiting daily temperature maxima greater than 25°C post anthesis. In this project, constitutive expression of LMA in semi-dwarf backgrounds has been demonstrated under controlled environment conditions based around limiting daily temperature maxima to below 25°C. Preliminary data suggest that this would be consistent with LMA levels resulting in low Falling Number readings in early sowings of field trials at Waite, South Australia (SA) and Esperance, Western Australia (WA). Controlled environment phenotyping could be an effective and lower-cost option for assessing the LMA risk of potential new varieties in the future. Finally, considerable progress has been made towards a better understanding of the mechanisms involved in LMA. This information will underpin efforts in continuing LMA projects to identify the genes underlying LMA QTL and development of gene-based diagnostic markers.

Recommendations
A number of initiatives have been discussed with GRDC over the past two years and these have been incorporated into new LMA projects, UA00150 and UA00153 that run until June 2018.
Under the current Wheat Quality Australia (WQA) classification guidelines, new varieties with LMA levels similar to Wyalkatchem are deemed eligible for release. Current varieties that fall into this intermediate LMA class have given low Falling Number in early-sown field trials in SA and WA. It would be very prudent for WQA and the wheat industry to maintain a watching brief, particularly in the higher risk regions identified in the early Queensland Alliance for Agriculture and Food Innovation (QAFFI) climate risk model.

Outcomes

Australian wheat breeding companies will benefit from this project through access to significantly better screening methods that include use of controlled environment phenotyping, as well as genotyping based on high-throughput molecular marker technologies free from the strong effects of the environment. In particular, genotyping should allow early generation selection that is not possible with phenotyping and lead to a reduction in the proportion of breeding lines proceeding through to the high input cost, later stages of the breeding programs. A significantly improved phenotyping protocol currently in use provides consistent data that is used in classification of new varieties and which enables the wheat industry to greatly reduce the risk of LMA impacting wheat growers and grain traders.

Achievements/Benefits

Phenotyping

More than 2,500 advanced breeding lines have been screened for LMA and the data used for variety classification. Frequency of LMA was very high in all what breeding nurseries in 2011, 2012 and 2013. Constitutive expression of LMA, i.e. without a cool temperature shock, was achieved in controlled environments when plants were grown at 23°C day/15°C night provided disease and moisture stress was absent. Similarly, good discrimination between +/- LMA genotypes was achieved in glasshouse trials in the Plant Accelerator (PA), diurnal temperature profile, maximum 24°C and minimum 15°C. LMA was expressed in the earlier sowings of time-of-sowing trials in Esperance, WA, and Adelaide, SA. Populations involving LMA sources and three resistant genotypes have been phenotyped for genetic analysis.

QTL discovery and marker-trait linkage analysis. The major LMA QTL for susceptibility on chromosome 7BL that explains up to 30% of the genetic variation has been validated in Cranbrook, Seri, Super Seri, Kennedy, ChuanMai18 (Rht8 source), BD159, RAC655, Lerma52, Reeves and a primary synthetic, AUS33402. Other QTL located on 3B, 3A, 2D and 6B are specific to particular genotypes. Conversely, resistance on 7B has been identified in Maringa and Chinese Spring in addition to Halberd . Interestingly, Hartog, possibly Janz, and Kamilaroi carry marker alleles normally associated with LMA on 7B. Selections from Halberd/Hartog, Maringa/Hartog and RAC655/Hartog RILs with Hartog alleles at the 7B QTL segregated roughly 50:50 for LMA, clearly showing not only that a ‘suppressor’ QTL located elsewhere on the genome was inhibiting expression but also the non-LMA genotypes carrying this ‘suppressor’ can give rise to LMA when crossed with non-LMA genotypes that do not carry the ‘suppressor’. This is being investigated further in UA00153. One population, Cleo-Inia(LMA)/Halberd, showed phenotypic variation similar to other populations mentioned above, but this variation was not associated with markers located on chromosome 7BL and warrants further investigation.

Validation of QTL and relative impact of individual QTL

The effects of different QTL rank in order 7B > 3B =3A > 2D. Comparison with other sources of LMA suggested that the effect on phenotype of the 7B QTL from Spica or Cranbrook were similar to the 7B QTL from ChuanMai18 (China), Seri (Mexico) and a synthetic hexaploid (Mexico). Interestingly, in the Spica/Maringa (rht) population the means for sets of lines with only 3A or 2D were not significantly different from the non-LMA control but appeared to be additive with 7B. The 3A/2D combination gave significant LMA.

Fine mapping of the 7B QTL

Durum sources of a non-LMA were identified amongst landraces carrying functional Bo1 alleles. Haplotype analysis indicated high similarity to Halberd across the critical 7BL region, suggesting a conserved close linkage between Bo1 and the ‘Halberd’ non-LMA trait. A durum origin for the Bo1 allele in Halberd (via the old variety Currawa) was recently determined. A range of new SNP and gene-based markers useful for tracking the Halberd 7BL locus have been developed. A number of candidate genes including 2 CPS (coding for an enzyme that functions in the early steps of gibberellin synthesis) and an Myb transcription factor have been identified for further study in UA00150 based on bacterial artificial chromosomes (BAC) sequence data from colleagues in Norway. This line of research has been adversely impacted by the presence of the durum
segment that seriously limits recombination with the corresponding chromosome segment in bread wheat. The non-LMA parent Maringa also appears to contain a segment of alien genome, probably durum, near the critical LMA QTL region. As a consequence, work on fine mapping the 7BL QTL has been slow and focus has recently shifted to use of Chinese Spring as the non-LMA parent. More than 350 F5 lines of RAC655/Hartog have been developed for LMA resistance QTL mapping in addition to 250 Kamilari/Yallaro recombinant inbred lines (RILS).

Fine mapping in the LMA QTL region on chromosome 3B and 3A

The 3B LMA is present in Cranbrook, Seri and BD159 but not Kennedy©, Cleo-Inia, ChuanMail8, RAC655 or a number of synthetics. Fine maps of the 3B LMA QTL have been produced for the BD159/CxH93, Seri/Halberd and Cranbrook/Halberd populations. Three new simple sequence repeat (SSR) markers significantly linked to the 3B QTL, two of which (gpw3134, gpw7148) are located in the region of highest logarithm of the odds (LOD) score, were co-located with the previously identified marker gpw1107 in the BD159/CxH93. 250 SNPs linked to the 3B QTL were identified in Cranbrook/Halberd and BD159/CxH93. 31 of the most closely linked markers have been successfully converted to KBioscience competitive allele-specific polymerase chain reaction (KASPar) markers.

In addition, seven SNP-based KASPar markers have now been added to the LMA 3A QTL map for Spica/Maringa. Due to the centromeric location of these QTL, recombination is poor and the DNA intervals are still relatively large, containing a lot of genes.

Mechanisms

Microarray data and hormone profiles during grain development suggest that LMA is associated with dramatic changes in gene expression and plant hormone content that coincide with the onset of the aleurone's capacity to respond to gibberellic acid (GA). The timing of the onset of aleurone sensitivity to GA did not appear to be different between LMA and non-LMA material and evidence for an increase in sensitivity following cool shock was inconclusive. Cool shock did maintain the level of GA species at a high level for longer although the significance of this observation remains unclear. GA contents in non-LMA controls were below the level of detection.

The major GA species, GA19, GA24 and GA44, identified in LMA affected grain are reported to be biologically inactive but their presence does indicate a significant activation of the gibberellin biosynthetic pathway. Novel GAs, GA54 and GA55, reported in the older literature on wheat grain are reputed to have some biological activity but could not be identified due to the lack of authentic standard compounds. This research will continue in UA00150 and collaboration with a colleague in the chemistry department has been set up with the aim of synthesising these novel gibberellins.

More recent results are difficult to reconcile with control of alpha-amylase synthesis in LMA via the published GA transduction sequence described for alpha-amylase synthesis in ripe wheat and barley grain during germination. The LMA semi-dwarf variety RAC655 displayed the hormone profile typical of LMA types but interestingly, apart from maintaining the level of GA at a high level for longer, was not altered significantly by the cool temperature shock that was generally used for expression of the LMA phenotype. Hartog, a non-LMA variety, showed the same hormone profile but no LMA phenotype even after a cool shock. Furthermore, genotypes with LMA associated with minor LMA QTL did not show a significant build-up of GAs. LMA was expressed in the Rht3 (extreme insensitivity to GA) isolate of Huntsman but not the Rht3 isolate of Nainari, whilst application of a GA synthesis inhibitor to LMA genotypes 10-15 days after anthesis inhibited the build-up of GAs but not development of the LMA phenotype.

High pl alpha-amylase genes, of which there appear to be a large number of copies, were expressed for only a few days preceding amylase protein synthesis, whilst neither glucanase nor a protease (typically activated during germination) were expressed. Assays of endo-protease found no evidence of protease synthesis.

Effects on quality

LMA had no adverse effect on surface stickiness of Asian noodles (texture analyser). However, LMA showed strong negative correlations with amylase, Falling Number, rapid viscosity analysis (RVA), dough stability and breakdown with less significant effects on bakers. The effects on farinograph dough stability and breakdown appear to be correlated with the level of LMA but are clearly not due to increased levels of endo-protease.

LMA in synthetics
Fifteen synthetics based on the durum Altar (very low or non-LMA) were compared with sets based on the LMA-prone durums Croc and GAN and synthetics produced at the University of Adelaide by crossing *Aegilops tauschii* onto Kamilaroi (non-LMA) or Yallaroi (LMA). All synthetics were tall, despite the use of Rht1 semi-dwarf durums, and most had a high LMA phenotype. In addition to LMA 7B, synthetics appeared to contain independent QTL on 6B with opposing effect on LMA. Synthetics have been identified and have been crossed with +/- LMA tall genotypes to determine the impact of these QTL.

**Intellectual property summary**

Release of KASP markers for LMA 7B, 3A and 3B limited to Australian wheat breeding companies following consultation with GRDC. Marker information provided to all companies simultaneously by email and receipt of all files confirmed by a signed ‘chain of custody’ statement.

**Additional information**


