Agricultural/Horticultural Science Research Studentships
Harper Adams University College

Research Studentship 1

1. **Title of Project**

Understanding and regulating pre-maturity alpha-amylase activity in wheat to maintain high Hagberg falling number through improved varieties

2. **Overall Aim**

To investigate the mechanism by which pre-maturity alpha-amylase (PMA) forms in wheat grain and investigate the potential of altering hormone sensitivity to regulate this process.

3. **Specific Objectives**

1) To determine the ABA and GA sensitivity of developing grain on intact plants and of aleurone layers isolated from PMA-induced and non-induced grain.
2) To assay endogenous ABA and GA concentrations in grain from plants induced to produce PMA and from non-induced grain.
3) To assess the impact of increasing ABA sensitivity and GA insensitivity in developing grain on PMA induction.

4. **Project Summary**

Low Hagberg falling number (HFN) is an intermittent cause of serious economic loss to growers of bread, biscuit, distilling and export wheat and to millers. There are two main causes of low HFN: pre-harvest sprouting (PHS) and pre-maturity alpha-amylase (PMA). A LINK project funded by BBSRC/DEFRA/HGCA and the plant breeding industry was initiated in 2006 and has made substantial progress in developing a controlled environment screen to reliably induce PMA. This knowledge is now being used in the HFN LINK project to screen large numbers of lines produced by the industrial partners to enable chromosome mapping of the quantitative trait loci (QTL) responsible for PMA. This will allow the development of DNA markers to be used in marker-assisted breeding for the future selection of lower PMA varieties.

In addition to QTL mapping, the HFN LINK project will produce a shortlist of candidate genes based on knowledge of gene function and on co-alignment with QTLs to identify gene-based markers that allow selection for reduced PMA. To date, the most promising candidate genes relate to either abscisic acid (ABA) or gibberellin (GA). This studentship will extend the HFN LINK work to further validate the role of ABA and GA in the induction of PMA. The student will also undertake the first investigation into the effectiveness of altering sensitivity to the two hormones as a means of reducing PMA. By comparing the effectiveness of altering ABA vs. GA sensitivity this work will inform future commercial selection programmes.

This studentship will use the screen to examine the role of ABA and GA in PMA induction. Preliminary experiments have shown that application of ABA to developing grain in intact plants reduces the occurrence of PMA while the application of GA increases it. The student will extend these findings by investigating the effect of PMA-

induction on both the endogenous ABA and GA levels and on the sensitivity of isolated aleurone layers to the two hormones.

The potential for hormone sensitivity to regulate the occurrence of PMA will be further assessed using two transgenic wheat lines. These transgenic lines are currently being developed as part of the HFN LINK project, by the industrial partner Biogemma. They will be available for testing by the end of 2009, but cannot be fully evaluated within the HFN LINK project which ends in March 2010. The first line is being developed to be hypersensitive to ABA by silencing the *ABH1* gene using RNAi; while the second is being developed to be hyposensitive to GA by silencing the *GID1* gene using RNAi. The student will use the screen to assess the impact of the altered sensitivity on the occurrence of PMA.

The scientific outcome will be a clearer understanding of the role of hormonal factors in the occurrence of PMA. The practical outcome will be an assessment of the potential for reducing PMA by genetic regulation of hormone sensitivity in the grain; in particular, this work will add value to the outputs of the HFN LINK project by validation of the use of gene-based markers for ABH1 and GID1 in breeding for low PMA.

3. Scientific Justification

Previous work on PMA has been hampered by the fact that the syndrome is typically identified in mature grain by which time the underlying causes are no longer present. The environmental stimulus for induction of PMA in wheat grain has now been clearly identified as a cold-shock applied from 550 to 750 degree days after anthesis (HFN LINK Work package 2 at HAUC). This provides a context and a timeframe with which to assess the mechanisms leading to PMA.

The mechanism by which the cold-shock induces PMA is not understood, but evidence suggests that the hormones ABA and GA are of central importance. GA initiates the synthesis of alpha-amylase in the aleurone layer (inner part of the bran), and ABA antagonises the action of GA. ABA and GA are known to control alpha-amylase synthesis in pre-harvest sprouting (the other main source of low HFN) and our preliminary experiments have confirmed that exogenous applications of the hormones can regulate the occurrence of PMA. The HFN LINK project has provided a suite of biochemical and molecular techniques for assessing the impact of these hormones on alpha-amylase production. The main hypotheses to be tested in this project are that either concentrations of ABA and GA or sensitivity of aleurone layers to ABA and GA change in response to the inductive environment in favour of GA.

This rationale is combined with a genetic approach to reduce PMA through the manipulation of genes that alter sensitivity to ABA and GA. Two transgenic lines will be tested: the first developed to be hypersensitive to ABA by silencing the *ABH1* gene using RNAi; the second developed to be hyposensitive to GA by silencing the *GID1* gene using RNAi. In both cases the suppression of the target genes will be restricted to the developing grain by use of the *VP1* promoter. The student will use the screen to assess the impact of the altered sensitivity on the occurrence of PMA. Such a comparison will greatly strengthen the scientific impact of this work and will provide an assessment of the potential for reducing PMA by breeding varieties with altered sensitivity to one or both of these hormones.

The work will be collaborative between HAUC, Rothamsted Research (RRes), Queen's University Belfast (QUB), Biogemma and RAGT Seeds Ltd. The student will be registered at HAUC and will conduct glasshouse and/or controlled environment experiments at HAUC (Objectives 1 and 2). The student will undertake analysis of endogenous hormones in grain at RRes (Objective 2). Biogemma will supply seed of the transgenic lines as an output from HFN LINK, and the experiments using these lines will be conducted in containment facilities at RRes (Objective 3). QUB will provide additional supervision based on expertise in developing PMA screening. RAGT Seeds Ltd will give advice on application to breeding, and supply seed of PMA resistant and susceptible varieties and advanced lines from their breeding programme.

4. Benefits of the project to the industry

Low HFN is an unpredictable cause of serious economic loss to growers of bread, biscuit, distilling and export wheat and to millers. Deploying every strategy to combat this problem is necessary to reduce these losses and to reduce annual volatility in the premium.

This project will complement and add value to the HFN LINK project by providing a clearer understanding of the mechanisms leading to PMA, and by validating two of the candidate genes selected in HFN LINK. This will provide clear guidance to commercial plant breeders of the potential of marker-assisted selection for either increased ABA sensitivity or reduced GA sensitivity in development of PMA resistant varieties. The involvement of RAGT Seeds Ltd will ensure that the work will focus on integrating the science with its application to breeding, and will also have a potential route to market.