September 2011
Cost £11.06

Project Report No. 480

An integrated approach to stabilising Hagberg Falling Number in wheat: screens, genes and understanding

by

J. E. Flintham[1], M. J. Holdsworth[2], P. L. Jack[3], P. S. Kettlewell[4] and A. L. Phillips[5]

[1]John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH

[2]University of Nottingham, Sutton Bonington Campus, Sutton Bonington, LE12 5RD

[3]RAGT Seeds, Grange Road, Ickleton, Essex, CB10 1TA

[4]Harper Adams University College, Newport, Shropshire, TF10 8NB

[5]Rothamsted Research, Harpenden, Herts, AL5 2JQ

This is the final report of a 54 month project (RD-2005-3196) which started in January 2006. The project was a Defra and BBSRC sponsored LINK project (SA326) costing £2.2 million, of which HGCA provided £160,000. Industry partners were Campden BRI, Biogemma, Elsoms, KWS, Limagrain, nabim, RAGT, SWRI and LM SW Seed.

CONTENTS

1. ABSTRACT

Hagberg Falling Number (HFN) is the wheat quality characteristic which is least amenable to agronomic or post-harvest manipulation and thus almost totally reliant on breeding for improvement. It is, however, difficult to breed for stable HFN and also difficult to assess HFN stability in the Recommended List variety trials due to the highly weather-dependent variation in HFN inductive conditions from year to year.

The main aim of this LINK project was to provide the information to the breeding companies to enable the technique of marker-assisted breeding to be implemented for HFN. A secondary aim was to establish the genetic and molecular mechanisms underlying the two principal causes of low HFN: pre-harvest sprouting (PHS) and pre-maturity amylase (PMA).

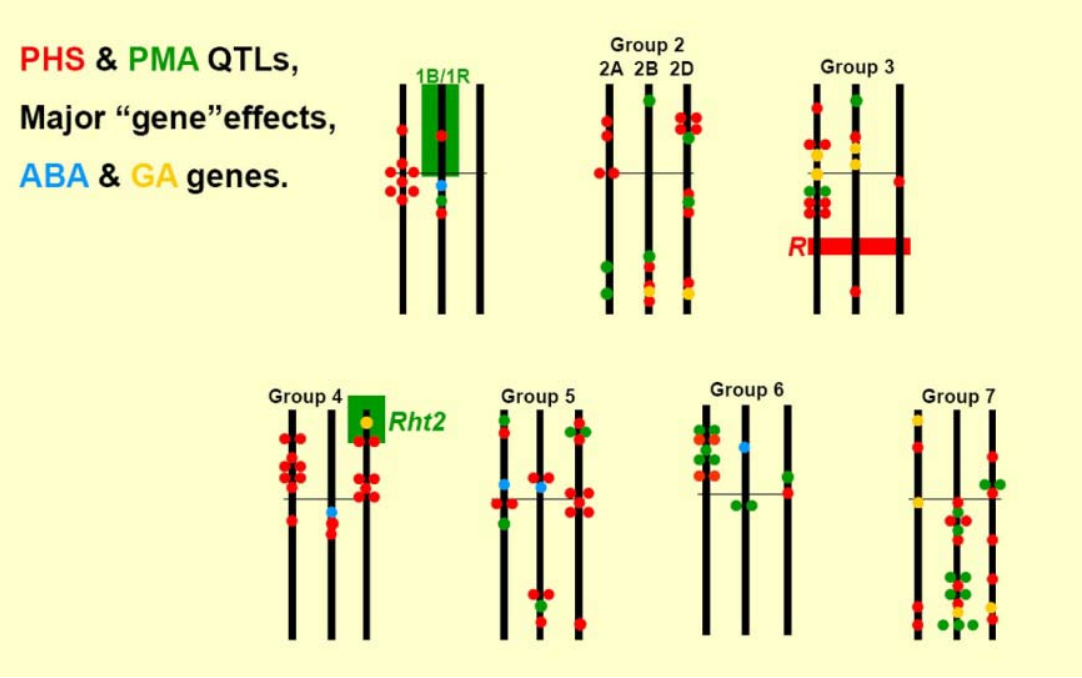
At the heart of the project was a series of field trials with mapping lines derived from several crosses between resistant parent varieties and susceptible parent varieties for PHS and for PMA. Some of these trials were overhead-irrigated to ensure PHS induction, and some were unirrigated to assess PMA if inductive weather occurred for PMA and not PHS. The position of genes controlling PHS and PMA (mainly quantitative trait loci [QTLs], each having a small effect) were located along the length of specific chromosomes by mathematical analysis following HFN measurement of grain from the mapping lines. The QTL locations were validated through the development of controlled environmental screening procedures for PHS and PMA and their use with selected mapping lines. The foundation for further developments in breeding for stable HFN was laid by identifying and mapping genes regulating hormones involved in amylase production in the grain. Understanding of PMA was enhanced by several cell biology and molecular techniques, which demonstrated that different varieties may develop PMA in different locations within the grain.

This project has provided, for the first time, a comprehensive understanding of the genetics of HFN in UK breeding material and this will enable varieties with more stable HFN to be commercially available within the next five to ten years.

2. SUMMARY

2.1. Introduction

Hagberg Falling Number (HFN) is a measure of the α-amylase content of flour made from harvested grain and is a major quality trait in wheat. HFN is sensitive to a number of environmental conditions that reduce the quality of grain, resulting in severe financial losses to growers and increased imports of grain for breadmaking. The increased unpredictability of weather conditions associated with global climate change suggests that low HFN will be an even greater problem in the future. The aim of this multidisciplinary project was to investigate the genetic and biochemical basis of the two major causes of low HFN in the UK: pre-harvest sprouting (PHS) and pre-maturity amylase (PMA). This very successful project has identified multiple genetic loci in UK bread wheat cultivars that confer resistance or susceptibility to PHS and PMA (Summary Figure 1), which will allow breeders to develop more resistant varieties. In addition, improved understanding of the molecular basis of PHS and PMA and characterisation of candidate genes will help to identify and validate these genetic loci. The project was divided into five workpackages as detailed below.



Summary Figure 1. Summary of QTL and candidate gene locations on the wheat genome.

2.2. Work Package 1: Physiological analysis of wheat seed dormancy and pre-harvest sprouting

Pre-harvest sprouting (PHS) is a major limitation to stability of wheat production in parts of the world where cool damp conditions prior to harvest are a possibility, and in the United Kingdom is the major cause of increased α-amylase hydrolytic enzyme activity in damaged seed lots. There is, therefore, a need to breed for increased resistance. PHS can be combated in part through manipulation of grain colour via the Red grain (R) locus that provides some resistance to sprouting. However, an inability to easily screen for this trait has hampered progress towards stable uniform wheat seed quality. Many studies have analysed the genetic control of PHS. One promising approach has been based on the observation that high PHS susceptibly is inversely related to high seed dormancy at harvest. Several Quantitative Trait Loci (QTL) associated with PHS have been identified. Most notably QTL on chromosome 4A that influence dormancy level at harvest and abscisic acid (ABA) sensitivity and PHS susceptibility have been found by several different researchers.

Key questions associated with understanding the phenomenology of PHS are the relationship between seed dormancy loss, measured in intact ears and the development of dormancy and sensitivity to the phyto-hormone abscisic acid (ABA) within caryopses during grain development, and how after-ripening affects dormancy capacity within the embryo. In this project we analysed the relationship between dormancy, after-ripening and ABA responsiveness in wheat seeds and isolated embryos. We showed that genetically-related varieties showing different susceptibilities to PHS demonstrate distinct kinetics of after-ripening, and that ABA responsiveness of embryos from these varieties is not directly related to after-ripening behaviour. We identified a positive correlation between speed of after-ripening and susceptibility of either intact or isolated ears to PHS. These resultssuggest that although ABA is a key determinant of dormancy induction during grain development, timing of after-ripening may be more closely associated with capacity of ears to withstand adverse weather conditions and maintain resistance to PHS.

A QTL approach was undertaken to identify genes that promote after-ripening using varieties identified with highly contrasting dormancy and after-ripening behaviour. This analysis identified a very strong QTL on chromosome 4AL, mapped to a region of only 4 centi-Morgans (cM). This QTL represents an exciting target for gene cloning in future work, to provide wheat breeders with a molecular marker to distinguish dormant and non-dormant wheat types.

2.3. Work Package 2: Pre-maturity alpha-amylase smart screen development

Pre-maturity alpha-amylase (PMA) is the most significant of a number of syndromes that result in high α-amylase levels in grain and low Hagberg Falling Number (HFN) in the absence of germination. PMA appears to be under different genetic and environmental control to pre-harvest sprouting (PHS). QTLs associated with variation in germination and PHS do not closely co-segregate with those few loci tentatively identified for PMA, and wheat varieties that have good resistance to PHS may be susceptible to PMA and *vice versa*. Similarly, the environmental conditions that give rise to PHS and PMA appear to be different. PHS is induced by a combination of warm temperatures late in grain development, producing low levels of dormancy, and rainfall around harvest time that promotes premature germination.In contrast, a range of environmental triggers have been shown to induce PMA-like symptoms: for example, transfer to low temperature, transient treatment with high temperature followed by low temperature at high humidity, and growth at lower temperature with high watering rates. The identification of reliable conditions for promoting and predicting PMA in susceptible lines was a key objective of Work Package 2.

In the first two years, a series of experiments was undertaken to compare published putative inductive conditions for PMA and the most appropriate was selected for use in a high throughput ‘smart’ screen. The screen was then used to phenotype mapping lines in the third year. The series of experiments was conducted in controlled environment cabinets and focused on testing published Australian work showing that transfers to a cool environment induced PMA. The experiments involved transferring plants of parents of mapping lines known to differ in susceptibility to PMA from a warm cabinet to either a cool or hot cabinet for a short period during grain growth, followed by α-amylase assay of grains using an antibody assay.

In the literature there is some evidence of a link between grain size and PMA, and this was evaluated by a statistical analysis of data on grain weight and HFN from UK Recommended List variety field trials obtained from Crop Evaluation Ltd. An experimental approach of manipulating grain size by degraining to artificially increase assimilate supply and grain size as a possible alternative PMA screening technique was also explored. Because ofthe volume of plants needed for the screen, four screens of Spark x Rialto and Option x Potent mapping lines were conducted in a glasshouse which included a purpose-built air-conditioned bay.

The results of the transfer experiments showed that both hot and cool transfers can induce PMA in UK germplasm, with one particular cool transfer regime found to be the most effective and repeatable. This cool transfer protocol was adopted for the smart screen and applied to mapping lines. The screens enabled a major gene affecting PMA to be identified, and data from the screens

has been used in Work Package 5 to map the location of PMA QTLs and to validate markers from field QTL phenotyping experiments.

Ultimately, this information will be used by breeders in the consortium to avoid taking to market varieties susceptible to PMA.

The grain weight analysis using variety trial data confirmed that a general link existed with heavier grains tending to have lower HFN both within and across varieties. The degraining experiment was successful in inducing larger grains, but PMA did not increase. This indicates that there may be genetic linkage between grain size and PMA, but not necessarily a mechanistic relationship.

In conclusion, Work Package 2 successfully achieved the aims of defining the inductive conditions for PMA in UK germplasm, and developed this into a high-throughput screen to augment field-based phenotyping of mapping lines.

2.4. Work Package 3: Molecular characterisation of pre-maturity alpha-amylase

This workpackage investigated the location of α-amylase in the grain in PMA-susceptible varieties, the role for gibberellic acid(GA) signalling in PMA and investigated the changes in gene expression during the induction process. As in the late-maturity amylase (LMA) syndrome identified in Australian varieties, PMA was shown to be an extremely stochastic process, with only a few grains in induced ears containing high levels of α-amylase. In Maris Huntsman, in which PMA appears to be constitutive, α-amylase was shown to be mainly associated with the transfer aleurone in the crease region of the grain. In Rialto, in which PMA is induced by cold temperature, the α-amylase enzyme is mainly found in the peripheral aleurone in the cheeks and dorsal surface of the grain. This suggests that different PMA syndromes exist in the two varieties. It was also shown that PMA symptoms can be reproduced in Cadenza by the overexpression of *GA20ox* in developing grain, presumably through increases in the levels of bioactive gibberellin; however, transcript analysis of candidate genes in the GA biosynthetic pathway in cold-induced PMA grain did not reveal any significant change in expression, nor were any changes in GA levels detected. Transcript profiling using microarrays identified differences in the cold response of PMA-susceptible (Rialto) and resistant (Spark) varieties. Further work will be required to examine whether such differences in the response to cold determine sensitivity to PMA.

2.5. Work Package 4: Identifying candidate genes for increased HFN stability in wheat using a post-genomics comparative approach

Candidate wheat genes with possible roles in regulating PHS/PMA were identified by investigation of model systems using sequence similarity and comparative transcriptome analyses. Possible roles for these candidates in wheat were defined via transgenesis, genetic mapping, analysis of novel variation in wheat and transcriptome analysis. Transgenic wheat lines containing suppression of candidate genes were produced and will be analysed within a follow-on project. Candidate genes from the GA (gibberellic acid) and ABA (abscisic acid) pathways were mapped onto the wheat genome, a small number co-locating with PHS/PMA resistance QTL. For the QTL on chromosome 4A identified in Work Package 1, TILLING (McCallum *et al.*, 2000) was used to identify mutants in a *GA20ox1* candidate gene from an EMS-induced population, and these are currently being back-crossed to remove excess mutations prior to characterisation for PHS responses.

2.6. Work Package 5: QTL Identification, validation and relation to candidate genes

Work Package 5 was designed to identify quantitative trait loci (QTLs) controlling HFN under conditions promoting PHS or PMA. PHS trials were conducted in winter-sown field plots which were irrigated from overhead during grain maturation, while PMA was investigated through its effect on HFN in non-irrigated plots.Experiments were repeated at different sites and across several years and on multiple mapping populations. A large number of resistance loci were identified, widely distributed across the wheat genome, indicating abundance of genetic variation. Transgressive segregation was observed more often than not, reflecting dispersal of resistance alleles among the mapping parents.

Resistance to PHS and PMA is determined by aggregate effects of major genes and multiple QTLs, but loci controlling PHS do not generally co-locate with PMA effects; the two syndromes are controlled by separate genetic mechanisms. It was found that both PHS and PMA exhibit moderate to high heritabilities, depending on seasonal variation in the severity of damage, but Genotype x Environment (GxE) effects were also prominent, affecting some loci more than others. Candidate genes are already known for the two strongest genetic effects controlling HFN (*Rht* and *R*). This study has furnished DNA markers for further investigation of other important QTL effects: for the development of novel marker technologies, for physiological characterization of their mechanisms, and for fine scale mapping and molecular analysis. There is, therefore, abundant potential for stabilising HFN in commercial wheat varieties for the future, using marker-assisted breeding to assemble novel PHS and PMA resistance gene combinations for more efficient deployment of existing elite germplasm.