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First Round Project Abstracts

Principal Investigator: Keith Edwards

Institution: University of Bristol

Reference Code: BB/I017496/1

Project Title: Development and validation of a flexible genotyping platform for wheat

Abstract: Developing new strategies to manipulate yield and pest and disease resistance by marker-assisted selection (MAS) underpins the UK's strategy to generate improved wheat varieties. Academic laboratories, genotyping service providers and breeding companies use MAS to track the inheritance of a host of loci controlling desirable traits such as disease resistance, drought tolerance and yield. Until recently most laboratories have used microsatellite markers in their MAS projects, while these markers continue to be used, for many species Single Nucleotide Polymorphisms (SNPs) have become the marker of choice due to their ease of use and scoring and their ability to be automated with relative ease. However, in allohexaploid wheat the task of identifying similar useful sequence polymorphisms is problematical due to the occurrence of homoeologs from the A, B and D genomes.

In this proposal, in collaboration, the John Innes Centre, IDna Genetics and KBioscience and Bristol University will develop both a wheat SNP database and a flexible genotyping platform for wheat. The utility of both will be validated by applying the tools and technologies to several exemplar projects.

If successful users will be able to access the technology via two routes:

The first consists of an in-house set-up requiring only basic molecular biology equipment (PCR machine and fluorescence plate reader). In this case, users would use the database to help them decide which assays to run and which primers/reagents (all publicly available through the database) to order and suggestions as to where these might be obtained. This constitutes a very low barrier for adoption as most users will be able to perform these SNP assays in house at the scale that best fits their purpose. This level of platform will be employed for the work carried out at JIC. The KASPar platform will replace JICs existing SSR genotyping service and, like the existing service, will be available for external work. An aliquot of the full set of KASPar primers will be kept at JIC and made available via this service.

The second form of access consists of outsourcing to competitive service providers, such as KBioscience and IDna Genetics. Both companies have high-throughput platforms installed which will help lower costs and both will be set up to run all the KASPar assays developed in this project. The user would only need to provide genomic DNA and a list of which assays to run.

In addition to KASPar, members of the community will be able to use the database to access a range of alternative genotyping technologies such as Illumina

GoldenGate/Infinium/SureSelect, etc. For instance, the database will enable researcher to either design suitable probes for ordering from the appropriate technology supplier or they will be provided with details as to who offers the required technology on a commercial bases. This database will then be updated until at least 2017.

Principal Investigator: Gareth Jenkins

Institution: University of Glasgow

Reference Code: BB/I017518/1

Project Title: Increased pest resistance in oilseed rape mediated by an enhanced UV-B response

Abstract: Attack by insects, molluscs and microbial pathogens causes significant losses to the oilseed rape crop and considerable expense is incurred in trying to counter this problem by using pesticides. The aim of this project is to undertake research to underpin the development of new oilseed rape varieties with increased resistance to insect herbivory. Exposure of plants to UV-B wavelengths, which are a natural component of sunlight, has been shown to reduce the attractiveness of plants to insect herbivores by altering plant chemical composition. Several plant responses to UV-B are mediated by a protein called UVR8 and the defence response to herbivory involves the regulatory molecule jasmonic acid. In this project, we will examine the potential of UVR8 and a protein called MYC2 that mediates the response to jasmonic acid to increase resistance to insect herbivory in oilseed rape. We will test whether transgenic over-expression of specific genes increases resistance

to insect herbivory as 'proof of concept' that manipulation of the UV-B response could be used to develop new varieties.

Principal Investigator: Lars Ostergaard

Institution: John Innes Centre

Reference Code: BB/I017232/1

Project Title: Exploring knowledge of gene function to combat pod shatter in oilseed rape

Abstract: Successful domestication of seed crops depends on the plant's ability to hold on to its progeny until harvest. The problem of seed dispersal control in cereals was solved thousands of years ago through simple selective breeding, but remains a serious issue for oilseed rape. The average annual loss experienced by farmers due to premature fruit opening, known as pod shatter is >10%. This loss can exceed >70% under particularly windy conditions, when wet weather delays harvest or if a hailstorm hits the field when the crop is ripe.

With an expanding human population and dramatic changes in climate patterns, the challenge to global food production has never been bigger, and to meet the demands it is essential that performance of our major crops be improved. However, the potential yield gain from such efforts cannot be fully realised if the farmer loses a significant part of his crop even before going out to harvest.

Fruits from oilseed rape dry out at maturity and open to allow their seeds to be dispersed in a process known as pod shatter. Unfortunately, all the fruits in the field do not dry out at the same time making it difficult for oilseed rape farmers to time their harvest and obtain all the seeds. In addition to a significant yield loss, the prematurely released seeds fall to the ground and germinate to become weeds (volunteers) and contaminate the harvest of the following year. This severely inhibits the crop rotation practice used by many farmers and is therefore also damaging to the environment.

Arabidopsis is a small, weedy plant that has been used as a laboratory model system to elucidate a wide range of aspects related to plant growth and development. Despite a dramatic size difference, fruits from Arabidopsis are remarkably similar to fruits from oilseed rape. In the past decade some of the key genetic regulators of fruit opening in Arabidopsis have been identified, and we have shown that these factors also function in species that are closely related to oilseed rape. In the proposed research project, we will exploit our knowledge to control pod shatter directly in high-yielding UK-elite oilseed rape varieties. Specifically we will manipulate and adjust the activity of a particular gene by the isolation of mutant plants and assessment of their performance in pod shatter-resistance tests. We will furthermore use mutated lines to expand our knowledge about the mechanism of fruit opening to enable us to fine-tune the pod shatter trait in future varieties. Particular strengths of this project are our genetic resources and our expertise in all the required technologies to successfully fulfil our objectives.

In conclusion, we believe that the presented project will provide substantial benefits for both farmers and consumers, as well as for the environment.

Principal Investigator: Alison Smith

Institution: John Innes Centre

Reference Code: BB/I017534/1

Project Title: Glucosidase inhibitors: new approaches to malting efficiency

Abstract: The brewing and distilling industries are of enormous economic importance to the UK. They have a major impact on farming because they use almost 2M tonnes of UK-grown barley (about one third of the crop, occupying one third of a million hectares of land) every year, they provide employment for tens of thousands of people, and their products are enjoyed not only in the UK but in many countries around the world. There is strong pressure on the industry to increase the efficiency with which barley grain is converted into beer and whisky. This is in part to maintain profitability, but also to reduce the production of waste and the amount of energy used in the conversion process.

The basic conversion process occurs in four main stages. First, during malting, the barley grains are soaked in water then allowed to start to germinate. Inside the germinating grain, enzymes are produced that can convert the starch stored in the grain to sugars. Second, during kilning, the grain is heated to dry it out so that germination stops. Third, the grain is milled then mixed with hot water. During this mashing process, the enzymes convert the starch to sugars. Finally, the sugar-containing liquid is drained off and yeast is added. The yeast converts the sugars to alcohol.

One of the major losses during the conversion of grain to beer and whisky occurs during malting. As soon as the enzymes are produced, they start to convert starch to sugars inside the seed, and the sugars fuel the growth of rootlets. Thus some of the starch store is lost before the mashing stage, reducing the potential yield of alcohol and resulting in the production of unwanted rootlets. This loss is between 5% and 10% of the starch. In the context of a market value of £20bn for the brewing industry alone, even a small reduction in the extent of starch loss during malting would have huge economic benefits.

Because of the economic importance of this malting loss, several different methods to prevent rootlet growth have been tested. However these have not been applied commercially, because of cost, toxicity, or adverse effects on the quality of the malt.

We have discovered that both rootlet growth and starch loss in germinating barley seeds can be reduced or prevented by the application of tiny amounts of natural plant products, called iminosugars. These products have the potential to reduce malting losses without undesirable side effects. Understanding how they work inside the seed will also provide new information that will help in developing better varieties of barley for brewing and distilling. In this project we will test natural products in a "micromalting" system that mimics real malting, and identify which ones are suitable for commercial trials. We will use biochemical and molecular methods to discover precisely how these products prevent the growth of rootlets, and the loss of starch. This information will enable us to identify genes in barley that are important in determining the malting quality of the grain.

To ensure that our research is relevant to the needs of the brewing and distilling industries, we will regularly consult an Advisory Panel that includes an expert on these industries, and also experts on barley grain germination, plant natural products, and malting

Principal Investigator: Martin Parry

Institution: Rothamsted Research

Reference Code: BB/I017372/1

Project Title: Manipulation of photosynthetic carbon metabolism in wheat to improve yield

Abstract: Ensuring food security is a major challenge given the projected need to increase world food production by 40% in the next 20 years and 70% by 2050 (FAO forecasts). There is clearly an urgent need to develop crop plants that yield higher outputs per unit area of land, without having to increase inputs of fertiliser or water. The aims of this research proposal are to test the hypotheses that: 1) The existing variation in photosynthetic capacity can be exploited to increase photosynthesis and total biomass of modern wheat cultivars; and 2) Genetic manipulation of photosynthetic carbon metabolism can lead to enhanced photosynthetic performance in crop plants and result in higher yields, increased nitrogen use efficiency and increased resource-use efficiency. We will screen existing wheat germplasm from a range of sources using a combination of carbon isotope discrimination, gas analysis and biochemical assays to identify plants with improved photosynthetic characteristics for use in future breeding programmes. We will also produce transgenic wheat plants with altered levels of SBPase and Rubisco. A detailed physiological and molecular analysis of the resulting transgenic lines will be carried out to determine the impact of photosynthetic parameters on growth in greenhouse grown plants.

Principal Investigator: John Walsh

Institution: University of Warwick

Reference Code: BB/I017410/1

Project Title: Exploiting sources of resistance to Turnip yellows virus for deployment in oilseed rape.

Abstract:

- The virus, Turnip yellows virus is found every year infecting most oilseed rape crops in the UK. In some crops, every single plant is infected. The virus has been shown to reduce the seed yield of oilseed rape crops in the UK by up to 30%.
- The virus is spread by greenfly and growers use insecticides to try to stop the greenfly spreading the virus to oilseed rape crops; the insecticides are not always particularly effective at stopping spread of the virus.
- We have identified a number of different plant lines with natural resistance to Turnip yellows virus.
- The different resistance sources we have identified will be evaluated against genetically different Turnip yellows virus isolates.
- The purpose of our research is to produce tools (markers) that will allow plant breeding companies to incorporate the natural virus resistance we have found in to their commercial oilseed rape varieties.

- Once the seed and markers have been handed over to seed companies, we will support them in incorporating / breeding the resistance in to their commercial oilseed rape varieties.
- The ultimate aim is to provide farmers with virus resistant oilseed rape varieties that will not become infected by the virus, or will tolerate some virus infection with minimal loss of yield.
- This will increase production whilst at the same time reducing inputs and energy costs / consumption. It will also reduce the farmers' dependence on insecticides and provide an environmentally friendly, sustainable means of increasing oilseed rape yields.

Second Round Project Abstracts

Principal Investigator: Anna Avrova

Institution: Scottish Crop Research Institute

Reference Code: BB/J019569/1

Project Title: Fungal effectors as activators of novel resistances in cereals

Abstract: This is a joint project between the James Hutton Institute (JHI, formerly SCRI) and Rothamsted Research (RRes) that focuses on the "Combating pests and diseases" research challenge highlighted by the industrial members of the Crop Improvement Research Club (CIRC). It is addressing one of the highlighted areas for the second CIRC call: Crop protection. Two teams with complementary expertise in different areas of plant science will combine efforts in exploiting pathogen genome sequence. We aim to advance fundamental understanding of plant immune responses and identify novel sources of resistance to the most economically important barley fungal pathogen *Rhynchosporium commune* (Rc), formerly known as *R. secalis*.

Rc can cause yield losses of up to 40% and reduce grain quality. Populations of Rc can change rapidly, defeating new barley resistance (R) genes and fungicides after just a few seasons of their widespread commercial use. New EU regulations may lead to loss of the most effective triazole fungicides, making Rc control even more problematic. All pathogens trigger non-host resistance (NHR) in plants. Successful pathogens can suppress or manipulate NHR by secretion of small proteins called 'effectors'. Once a pathogen has suppressed NHR, plants deploy a second layer of defence in the form of R proteins. R proteins detect certain pathogen effectors, termed 'avirulence' (Avr) proteins, and activate resistance responses. Pathogens can avoid recognition by some of the R proteins by losing either the expression or function of a non-essential (redundant) effector with no apparent cost to pathogen fitness. Both of these strategies have been deployed by Rc, mutating or eliminating AvrRrs1, to completely overcome Rrs1-mediated resistance in under 10 years.

We aim to understand redundancy within Rc effectors. R proteins recognising non-essential effectors are not durable. Therefore breeding should aim to target introgression of R genes

recognising essential effectors that are less variable in pathogen populations. This effector type has been found for other fungal and oomycete plant pathogens. Rc genome sequencing has provided a unique opportunity to identify the putative effector repertoire. Comparison of genome sequences of 9 Rc strains that are able to overcome different R genes will allow rapid prediction of candidate effectors that are less variable in Rc populations, and therefore are more likely to be indispensable. RNA sequencing of Rc germinated conidia and barley leaves infected with Rc provides important information about predicted effectors expressed during the onset of infection. Expression of less variable candidate Rc effectors will be assessed throughout the infection. Based on expression profiles, degree of conservation between the strains, and the ability to induce cell death in one or more barley genotype, 25 predicted effectors will be chosen for targeted gene disruption to identify those essential for fungal pathogenicity.

The RRes team has recently developed an efficient system for screening barley germplasm for recognition of Rc effectors. It is based on systemic expression of Rc small secreted proteins in barley leaves using a plant virus as a delivery vector. This method can be extended to other cereals, including wheat, and their pathogens. The extensive JHI collection of barley cultivars, landraces and mapping populations will be screened to (1) identify novel sources of distinct and potentially durable resistances to Rc, which can be combined to increase the durability of resistance, and (2) characterise resistance already present in current breeding material. This will have direct positive impact on Rc disease resistance breeding programmes. Deployment of this resistance will stably increase yield and quality of new barley cultivars, while reducing fungicide use, greenhouse gas emissions and environmental pollution.

Principal Investigator: Gary Bending

Institution: University of Warwick

Reference Code: BB/J019690/1

Project Title: Yield improvement of oilseed rape through genetic manipulation of rhizosphere exudation

Abstract: Plants use photosynthesis to fix CO₂ to sugars, which are used for plant growth and development. Up to 50 % of carbon (C) fixed by plants is moved to roots, where it supports growth of root systems through the soil, in order to take up nutrients and water and drive growth. Healthy growing roots pass a large proportion of the C they receive to the soil as 'rhizodeposits'. This includes a range of materials, but soluble exudates, consisting of organic acids (e.g. citric acid), carbohydrates (e.g. glucose) and amino acids (e.g. leucine) comprise the largest rhizodeposit component. The total amount of rhizodeposits, and the specific types lost from roots, vary between plant species and genotypes within species, and is influenced by plant developmental stage and environmental parameters. Because of rhizodeposition and the presence of readily available C, the soil surrounding roots has large populations of microbes, forming a distinct microbial niche, which is termed the rhizosphere. Rhizosphere inhabiting microbes interact with plants in many ways- some are pathogens or beneficial symbionts, and others are involved in cycling and transformation of crop nutrients such as nitrogen (N) and phosphorus (P), altering their availability to plants. These processes determine plant productivity even in agricultural systems in which fertilisers are added to maximise production. The types of organisms which grow in the rhizosphere are determined by the amount and types of materials plants exude.

In the current project we propose to elucidate plant genes involved in determining the amount and types of substrates lost from roots as rhizodeposits, and the response of exudation to plant development. Plants exude a very diverse collection of organic molecules and we will take advantage of cutting edge mass spectrometry techniques to understand the full complexity and composition of rhizodeposits for the first time. Additionally we will use newly emerging high throughput sequencing techniques to profile the genes expressed in roots and their association with release of rhizodeposits.

Furthermore we will investigate the way in which altering the composition of rhizodeposits influences the diversity and composition of microbes inhabiting the rhizosphere, and investigate how the function of these communities is influenced by varying exudate quality and quantity. Soil microbial communities are incredibly abundant and diverse, and we will use the latest high throughput sequencing techniques to characterise the microbial metagenome, allowing us to investigate all the major processes taking place in the rhizosphere simultaneously and the specific components of the community involved. Furthermore we will determine the consequences of rhizodeposition for crop yield, which will provide the first direct quantification of the benefit plants receive from rhizodeposition.

The work will open exciting possibilities to breed new crop varieties in which rhizodeposition is managed to enhance crop yields, increase agricultural sustainability and reduce environmentally damaging inputs. This work could lead to the identification of genes which could be used in breeding programmes for a number of applications, for example 1. Reduction of rhizodeposition could be used to increase C allocated to above ground growth, so that crop yields are enhanced. 2. Managing rhizodeposit quantity and quantity could be used to tailor exudation to promote solubilisation and uptake of growth limiting nutrients such as P, S, K and trace metals, and increase tolerance to harmful soil metals, particularly aluminium 3. The quality and quantity of rhizodeposits could be managed to engineer specific microbial communities in the rhizosphere. This could have many advantages, for instance inhibiting the growth of soil-borne pathogens which reduce crop growth, and stimulating communities which enhance the availability of crop growth limiting nutrients, such as P and N.

Principal Investigator: Andy Greenland

Institution: National Inst of Agricultural Botany

Reference Code: BB/J019496/1

Project Title: Production of wheat lacking B-type starch granules

Abstract: Starch is a major component of cereal grains and its functional properties have a significant impact on grain utilisation. Of considerable importance is the size and shape of the starch granules. In wheat, barley, rye and most of their wild grass relatives, there are two types of starch granules, called A- and B-type. These differ in size, leading to a bimodal granule size distribution that is unusual amongst plant starches and not found in other grasses, including Brachypodium, oats, rice and maize.

The smaller, B-type starch granules have negative impacts on many end-uses of wheat and barley. So far, attempts to reduce or remove B-granules from these crops by breeding have failed. The reason for this is the lack of genetic variation in B-granule content between cultivars. However, there is much greater variation for this character between species of *Aegilops* (Goat Grass), wild grasses that include the ancestors of bread wheat. The

existence of *Aegilops* species lacking B-granules suggests that it should be possible to introduce variation for B-granule content into the closely-related crop species.

The project builds on previous work in which we identified a major QTL controlling the content of B-type starch granules in *Aegilops*. Our ultimate goal is to identify and manipulate the gene responsible for the control of B-granule content in wheat and barley, *Bgc-1*. In this project, we will investigate gene order in the region of the genome harbouring *Bgc-1* and compare it with that in other grasses. If the opportunity arises within our project's timeline, we will be ready to use the latest developments from ongoing genomics projects in other labs to identify the orthologs of *Bgc-1* in wheat and barley and we will begin to manipulate *Bgc-1* in these crops using RNAi and TILLING technologies.

Prior to the identification of *Bgc-1*, we will start to produce mutant wheat plants lacking B-granules using a pre-existing collection of deletion lines of a breadmaking wheat cultivar Paragon. Previously, this population has been successfully used to generate wheat mutants with novel phenotypes by stacking deletions of genes. By screening for deletions spanning the *Bgc-1* region, we can select lines likely to lack *Bgc-1* in each of the three genomes of wheat and then stack these into a single plant by repeated rounds of crossing and selection.

This project will produce: 1) a fine map of the *Bgc-1* region and possibly identification of the *Bgc-1* gene in *Aegilops* and a comparison of this region/gene with those in other grasses. 2) near-isogenic lines of wheat and *Aegilops* that will allow functionality testing to determine the utility of B-granule-less grains. In addition, if *Bgc-1* is identified, the production of genetically manipulated lines of wheat and barley and/or TILLING mutants lacking B-granules will be underway by the end of the project.

Principal Investigator: Martin Broadley

Institution: University of Nottingham

Reference Code: BB/J019631/1

Project Title: Delivering low-cost, high-throughput root phenotyping screens for arable crops

Abstract: Plant roots are essential for the uptake of water and nutrients from soil. Consequently, root growth has significant effects on crop establishment and yield. Previous work by the project team, and others, has shown strong relationships between early root growth traits and the performance of arable crops in the field. However, measuring roots and selecting varieties with improved root systems in the field is time consuming, laborious and expensive. Using laboratory techniques, root growth can be measured quickly and cheaply - for 1000s of plants a year. Genotypes with better root growth and root architectures can be identified in the laboratory and assessments of selected plants can be made under field conditions to validate laboratory screens and assess field performance.

In this proposal we will use low cost, high-throughput methods to define the early root system of >1,600 different oilseed rape (OSR), barley and wheat genotypes in the laboratory. The roots of individual plants will be imaged at two time points. These images will then be analysed to determine the number of roots, root branching rates, root lengths, root growth rates and root angles. To validate and test the utility of measurements made in the laboratory, we will compare them with (1) measurements of root systems made in the field, and (2) data collected from new and legacy field trials assessing large numbers of new

crop varieties for National and Recommended Lists to identify root traits correlated with establishment and yield for breeding.

Root growth and architecture are genetically controlled. We will identify genetic loci in large populations of OSR, barley and wheat affecting root growth and architecture traits that correlate with resource acquisition, establishment and yield in the field. An understanding of how best to combine beneficial alleles will be assessed through modelling approaches. To identify genetic targets for breeding we will develop mathematical models describing root growth and architecture in OSR that incorporate the effects of genetic variation. These mathematical models will be extended to predict the effects of root architecture on P acquisition and, thereby, identify potential genotypes with improved rooting and greater P acquisition for sustainable agriculture.

In summary, this proposal will deliver low cost, high-throughput platforms for root phenotyping. These will be of direct benefit to the breeding industry, allowing them to assess germplasm for root growth and architecture that correlate with improved establishment and yield. Genetic loci affecting root growth and architecture will be identified to accelerate the breeding of new varieties. Mathematical models will allow genotypes associated with improved root systems to be identified.

Principal Investigator: Stephen Paul Hoad

Institution: Scottish Agricultural College

Reference Code: BB/J019623/1

Project Title: Causes and control of grain skinning in malting barley: Phenotyping and genetic analysis

Abstract: The quality of malting barley is of paramount importance, for reasons of food safety, product quality and the competitiveness of the UK cereals industry. Barley grains have an outer coat called a husk. Loss of the husk during harvest or post-harvest is called grain skinning. This undesirable condition has very serious consequences for farming and food sectors that depend on UK malting barley. Primarily, grain skinning is a serious problem in the malting process. However, its financial implications extend across the whole supply chain. Breeders invest about £2M in bringing a new barley variety to market, this spend is wasted if farmers and the malting industry no longer approve its use. Even low levels of skinning mean that loss of barley quality or malt production amounts to several £ million.

If in a batch of barley there are grains without husks, the malting process becomes very uneven as these grains will take up water and begin to grow (germinate) more rapidly than grains with firmly adhering husks. Sometimes, grains without husks sustain damage that prevents them from starting to grow (germination). This can give rise to mould growth. In grains with a loosely adhering husk, germination during malting tends to be more vigorous than in grains with a tightly adhering husk. This leads to handling problems and to greater malting losses. In brewing, the husk plays a vital role in filtration of the liquid that is produced from mixing the malted barley with hot water in the brewing vessel. Malting barley is, therefore, rejected by maltsters if it contains an undue proportion of skinned grains, with either no husk or an incomplete husk.

Weather conditions such as wet and dry spells during summer months appear to have a strong influence on skinning. However, so does the genetic make-up of different plant types (varieties). This means that an understanding of how a plant's genetic make-up influences grain skinning will increase the likelihood of breeding new barley varieties without this undesirable condition. Differential growth the husk and the underlying grain, or poor quality of the "glue" that bonds them together are likely causes of skinning.

Crop breeding supported by high quality science will help to solve this problem, as it will underpin the development of new barley varieties, with improved husk adhesion properties, and thus provide more reliable grain and processing quality for the UK cereals supply chain. Identification of plant screening and genetic tests will enable susceptible barley varieties to be eliminated before they are recommended for use by farmers and the malting industry. This will provide greater security for the UK barley supply chain and a more efficient development pipeline for the plant breeding of new varieties. An added benefit to farming is the promotion of more efficient use of inputs, as these will not be wasted on poor quality or rejected crops.

The aim of this project is to understand how differences in grain development, and their genetic controls, give rise to skinning. The outcome is for new varieties to be bred without this undesirable condition. The key areas towards application of this research are: (1) Understanding how weaknesses in husk and grain growth cause skinning; (2) Establish procedures to screen-out weak varieties, based on their grain characteristics (phenotyping), that give rise to skinning; (3) Identify the location of genes that influence or determine skinning; this means relating genetic locations to grain characteristics which lead to resistant and susceptible varieties; (4) Work towards the development of genetic (molecular) markers to identify 'good' and 'poor' varieties and thus eliminate weak varieties from being grown on farm and (5) The uptake of plant screening and genetic tests by crop breeders.

Principal Investigator: Peter Shewry

Institution: Rothamsted Research

Reference Code: BB/J019526/1

Project Title: The role of lipids in determining gas bubble retention in wheat dough

Abstract: Bread is an essential dietary staple, which has a significant influence on the nutritional profile of the population in terms of energy intake, fat and salt consumption. Approximately 80 million loaves are produced in the UK each week in a business worth around £2.5 billion per year. The UK still imports significant amounts of wheat for bread making due to higher protein content and quality. Bread quality is determined by gluten strength and dough bubble stability, which have impacts on loaf volume and crumb structure, respectively. The gluten network formed in dough controls the elasticity of the dough which in turn controls the dough's ability to rise during proving, and its behaviour during baking. Bubble stability controls the extent to which bubbles coalesce during this time, enabling the fine texture typical of UK sliced bread. However, while dough strength is now well understood, with plant breeders routinely selecting for specific gluten proteins which confer high dough elasticity, bubble stability is as yet still poorly understood. Therefore if we can identify ways by which UK grown wheat can be improved to give better bubble stability, we would enable:-

- breeders to develop better quality wheats,
- manufacturers to produce better quality bread,

- reduced reliance on imported wheat
- development of healthier bread with reduced salt and fat

The proposal focusses on how the gas bubbles in dough are stabilised. Gas bubbles can be stabilised by proteins, surfactants or lipids forming a stabilising layer on the surface. The molecular properties of the stabilising layer will determine whether the bubbles burst or coalesce. This is particularly important as the dough rises or proves, because to increase the volume of the loaf, the gas bubbles in the dough expand, and eventually come into contact. It is at this point when they will either remain stable, producing a good quality bread with fine structure, or the bubble will coalesce with each other, leading to partial collapse of the dough, and poor quality bread. Research has focused on both protein and lipid components in wheat flour, but the story is not clear, as dough is fragile but viscoelastic and therefore difficult to study directly without destroying the gas cell structure. However, the consensus is emerging that it is the wheat lipids largely control bubble stability.

Wheat flour contains a range of lipids, all of which will adsorb to the surface of the bubble, but their differing molecular structures will have different (positive or negative) effects on bubble stability. The lipid composition of the flour will therefore be critical for dough stability. Using state of the art lipid analysis techniques we will identify the lipids which stabilise gas bubbles in dough. Using novel surface and biophysical techniques we will determine how the different lipids stabilise the gas bubbles, and what their effect is on the stability of the dough and the quality of bread produced. We will determine the variations in the amounts of the different lipids occurring different wheat varieties to develop targets for breeders to improve the bread making quality of UK grown wheat.

In addition, improving the gas bubble stability in bread dough will allow manufacturers to reduce the levels of salt, fat and emulsifiers in bread. This is because salt is required to improve dough strength, and fat (as shortening) and emulsifiers are added to improve gas cell stability. Increasing the natural stability of the gas bubbles will reduce the need for the levels of salt and fat currently required to produce the quality of bread desired by consumers.

Principal Investigator: William Thomas

Institution: Scottish Crop Research Institute

Reference Code: BB/J019593/1

Project Title: Improving the processability of malting barley

Abstract: Of all the cereals, barley is grown over the most diverse environmental ranges as it is more tolerant of stress conditions. Malting for use in brewing and distilling is the major industrial usage with a demand of 20M t/yr, approximately 15% of world production. A much higher proportion of the crop is used for malting in the EU, e.g. just under 30% in the UK. World beer production is currently 1.85 billion hl and is thus by far the major consumer of malt. Production of malt and beer takes place on a large mechanised scale where systems are optimised to a target production cycle. Whilst plant breeding has improved the malt extract and thus the litres of beer that can be produced per tonne of malt, maltsters and brewers still encounter problem batches that do not process properly, e.g. because separation of fermentable liquid from residue solids can be slowed down or even halted by too much protein and/or cell wall residues. Such a situation causes production delays and incurs cleaning and residue disposal costs. Ease of processing (processability) is therefore second after malt extract on the Institute of Brewing and Distilling's 'wish list' of desirable

characters for UK malting barley. Processability problems are much more apparent in samples that are less than ideal for malting as even poor malting quality varieties give adequate levels of malt extract and process efficiently when grown under optimum conditions. As most malting tests are conducted from sites identified as producing good malting quality samples, processability problems generally appear once a variety has been recommended and grown on a larger scale. This is a waste of plant breeding and end-user time and resources and a strategy must be found to enable the selection and promotion of varieties that meet end-user needs under a wide range of environmental conditions. This target will become increasingly important to UK (and world) agriculture as climate change is likely to result in harsher and more variable environments for malting barley production.

We have considerable experience in the genetic analysis of economically important traits and have already amassed a range of performance (phenotypic) data upon a set of over 500 UK elite barley lines. In addition, we have DNA fingerprints of each of these lines and are experienced in combining such datasets in analyses designed to identify specific regions on barley chromosomes that are associated with differences in performance. We will select subsets of 100 spring and 100 winter barley lines from the 500 and grow them in trials under regimes designed to contrast for grain nitrogen content and thus provide contrasts in malt processability. By combining the processability data with the genotypic data, we will be able to associate regions of barley chromosomes that effect differences in malt processability. We will also sample RNA from a smaller subset of the lines in trial at stages when we expect the components affecting malt processability are being synthesised during grain development and degraded during germination and analyse the gene expression profile of each line at each stage. By selecting lines that are known to differ in processability, we can compare the overall pattern of 'good' lines with that of 'poor' lines to filter out genes that are being differentially expressed and thus are likely to be involved in the control of processability. The 50,000 genes that we will assay this way all have a known location on barley chromosomes so we can compare the results from the expression analysis with those of the association analysis to see if any co-locate. We will then compare the DNA sequences of genes from the expression analysis that co-locate with regions from the association analysis to detect if any sequence variants are associated with differences in processability. Results will then be tested through larger scale brewing tests and validated through analysis of a small independent panel of lines.

Principal Investigator: Richard Whalley

Institution: Rothamsted Research

Reference Code: BB/J01950X/1

Project Title: Phenotyping root function in wheat

Abstract: In the UK, approximately 30% of the production of wheat is on soils where insufficient soil moisture decreases yields by (on average) 1-2 t/ha. This costs between £112M and £224M each year in lost production (Foulkes et al. 2001). Studies comprising a limited range of wheat cultivars have shown that genotypes with deep root systems have been associated with high yield in water limited environments, while those with shallow root systems have been associated with increased nutrient uptake when soil water is plentiful. However, there is no comprehensive understanding of what configuration of root system architecture leads to improved resilience of yields, water and nutrient use efficiency, or what the trade-offs, if any, there are with yield potential. This is because roots are hidden

underground, and important traits are discovered only by laborious, destructive excavation of roots.

This project will develop a rapid, non-invasive technique using electromagnetic inductance (EMI) to measure the degree of soil drying at different depths in plots of different wheat varieties. Current EMI technology can be used to profile with changes in conductance with depth, but is not being used to study root activity. A key objective for the project will be to optimise the existing capabilities of EMI, so that they can be used to characterize water extraction profiles beneath different varieties of wheat. We will use electrical resistance tomography (ERT), which is labour intensive, invasive and slow, as a tool to provide high resolution images of soil drying to help with the optimisation of EMI which is rapid, non-contact and efficient.

Patterns of soil moisture extraction through the soil profile as the crop develops, which are related to growth and activity of the root system, will be measured with EMI. These data will be validated using conventional techniques such as root sampling via soil coring, buried soil moisture probes, changes in soil strength via penetrometer measurements, and root pulling strength. Initial field tests will comprise 20 UK elite wheat lines, some of which in preliminary data have shown differences in soil water extraction patterns. We will use our new root phenotyping tool kit in field trials with the Avalon x Cadenza mapping population, which has already shown significant genetic variation for nutrient uptake, yield and grain quality. We will determine the correspondence between QTLs identified with our new tool kit and wheat root QTLs already published. We will use soil drying data at various depths to test hypotheses that describe relationships between yield, deep water extraction, soil strength and root placement within drying superficial soil layers. This information is essential for the breeder: for instance, it is not enough to know which varieties can produce deeper roots; confirmation that such a root system translates to greater yield and yield stability across a range of environments is also required before any investment is made in selection for particular root traits.

At the start of the project we will establish a project advisory panel comprising breeders and other members of industry to help guide the selection of materials for investigation. This project will provide a completely new measurement possibility that can be applied to large field trials to give a spatial map of soil water at different depths over time. With the help of the project advisory panel we will identify existing field trials that can be used to test our new methods. There are numerous laboratory methods available for phenotyping roots in seedlings that have led to the discovery of QTLs linked to various root traits. However, it is rare that any of these QTLs are validated under field conditions because current methods of examining roots in the field are time-consuming and expensive. The proposed studies will fill this gap, and can possibly complement work on wheat roots in the BBSRC-LINK project based at NIAB.

Principal Investigator: Zoe A Wilson

Institution: University of Nottingham

Reference Code: BB/J019666/1

Project Title: Developing a Cereal Fertility Pipeline (CerFip) for wheat and barley

Abstract: There is increasing global awareness of the importance of agriculture and food security. Predictions of a 50% population increase by 2050 emphasise the urgent need for

sustainable, effective agricultural systems. Strategies for improved crop productivity without increasing environmental impact are critical. Selective breeding for key traits, combined with the use of hybrid lines has the potential to realise these goals. Hybrids are the progeny derived by crossing two distinct individual together. Hybrids tend to show "hybrid vigour", this can be seen in terms of increased growth, but also overall yield. Hybrid vigour results in the superiority of a hybrid over its parents, for example hybrid rice has 20-30% increased yield compared to inbreds as such hybrids are extremely valuable and in China hybrid rice constitutes >50% of all rice grown. However, the generation of hybrids is difficult and expensive, and has not been easy to achieve in temperate cereals, such as wheat and barley.

Emasculation is often needed to generate hybrids, because plants frequently self-fertilise before cross-fertilising, this is labour intensive, requires specific germplasm, or has high environmental impact. The slow development of hybrid temperate cereals is a reflection of the bottleneck in the availability of germplasm and understanding of traits controlling male fertility. Recently there have been reports of Hystar hybrid wheat (Syngenta) with yield increases of ~0.5t/ha and hybrid barley (Syngenta) showing >10% increased yield. However, these reports are restricted by a lack of molecular understanding of cereal reproduction. There is also a need for subsequent rescue of male sterile lines, therefore approaches involving inducible gene systems, or switches in fertility based on environmental sensitivity are needed. It also seems likely that environmental changes, such as low/high temperature may be impacting on pollen viability and therefore reducing yield.

A better molecular understanding of cereal pollen development that would facilitate effective control of male fertility would aid in increasing yield potential. Much of this knowledge of the molecular processes of pollen development has/is being developed in the model systems of Arabidopsis and Rice. This proposal will exploit this knowledge and utilise newly available techniques in wheat and barley genomic analysis to develop a Cereal Fertility Pipeline (CerFip) for trait transfer from model systems into temperate cereals for the control of male fertility. It will generate germplasm and genetic resources for the manipulation of fertility for selective breeding, maximal fertilization/seed set, and hybrid production, which are essential for yield improvement and food security. It will use comparisons between the different genomes to identify the corresponding genes in barley and wheat. These newly identified genes will be tested to confirm their role in pollen development and provide greater insight into male reproduction in temperate cereals. Germplasm will be developed to test selected genes for their application in switchable systems between male fertility and sterility. Such material will be potentially very valuable in the future development of systems to control fertility for hybrid development.