

It has been estimated that the demand on cereal production will increase by 50% by 2030; scientists and breeders alike must now look to understanding the genetic make-up of wheat to help maximise its growing potential.

Customer case study



University of Bristol – wheat genomics

Why wheat?

Wheat is one of the three most important crops for human and livestock feed, and with food supply an increasing global concern, the pressure is on to increase cereal crop production as a solution to feeding the growing world population.

It has been estimated that the demand on cereal production will increase by 50% by 2030; scientists and breeders alike must now look to understanding the genetic make-up of wheat to help maximise its growing potential.

Industry challenge: struggling to meet a growing demand

Compared with other crops, the increase in wheat yields

has slowed since the ‘green revolution’ of the 20th century due to the domestication of the crop.

This domestication of wheat has resulted in a decline of genetic diversity and it has been suggested that in order to increase it, genes from ‘wild relatives’ of the crop could be introduced. However such strategies often referred to as pre-breeding, can be resource intensive.

While diploid crops (consisting of two sets of chromosomes) such as rice and barley, have benefited from extensive genetic analysis and molecular breeding programmes, the complexity of a polyploid species such as wheat (more than two sets of chromosomes) coupled with a decline in genetic diversity has hindered these types of studies.

Aside from the challenge of meeting a growing global demand for wheat, the crop is also vulnerable to pests



and disease. For example, the stem rust pathogen known as Ug99 is one of the most serious diseases found in wheat and poses a significant challenge to production. To combat this, researchers need to identify genes within the wheat genome that can provide a strong and durable resistance; access to new screening technology is essential in defending it from this and other threats and to sustainably produce sufficient and safe food.

Technical challenge

The Cereal Genomics group, based in The School of Biological Sciences, University of Bristol, has recognised the need to better understand the complexity of the wheat genome in order to assist molecular breeding programmes.

Marker assisted selection (MAS) is a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) can be tracked through populations and used for indirect selection of genetic determinants of the trait of interest such as:

- Productivity
- Disease resistance
- Stress tolerance
- Quality.

MAS has a long history of successful use by academic laboratories, genotyping service providers and breeding companies for this type of genetic analysis in plant and animal breeding.

However, the complexity of the wheat genome coupled with a decline in genetic diversity within modern elite cultivars has made application of MAS in wheat breeding programmes a significant technical challenge.

A crucial step in the successful application of MAS in breeding programmes is the development of

cheap and easy to use molecular markers, such as single-nucleotide polymorphisms (SNPs). Until recently most wheat laboratories used microsatellite markers in their MAS projects. However, for wheat the new type of SNP marker is becoming more readily available.

This new approach has been adopted by wheat genome scientists at the School of Biological Sciences, University of Bristol, led by Professor Keith Edwards.

“Our aim is to identify tools and technologies to make it easier to do smart breeding to create new varieties of wheat. In particular, this would be used to improve yield and pest and disease resistance, including in commercial lines.”

The group has identified that in order to make wheat pre-breeding more efficient, the development of molecular markers capable of tracking any introduced genomic regions in large numbers of lines is vital. According to Professor Edwards:

“SNP analysis is straight forward and can be automated with relative ease but the task of identifying useful SNPs polymorphisms in wheat is problematic due to its complex and large genome.”

Molecular markers must be capable of distinguishing between the relatively large numbers of polymorphisms seen in homoeologous and paralogous genes, compared with the relatively infrequent varietal polymorphisms.



Homoeologous/paralogous and varietal SNPs have previously been studied and used in polyploid crops, however, these studies have also shown that distinguishing intervarietal markers from intergenomic polymorphisms is complicated and prone to error. These results, together with the large size of the wheat genome mean that despite the global importance of wheat, there are still relatively few validated varietal SNP markers in regular use.

The solution

Developing new strategies to enable UK wheat breeders to breed for yield and crop resistance by MAS underpins the UK's strategy to generate improved wheat varieties.

The development of SNP-based genotyping platforms has led to an increase in the number of protocols available for analysing genetic variation in numerous species.

Despite the inherent challenge of large-scale genotyping in polyploid species, which carries with it a high instance of homoeologous and paralogous genes, a number of platforms have recently been developed to perform high-density genotyping (large numbers of SNPs, with small numbers of individual plants); these have been successfully employed to genotype wheat.

These technologies can be difficult to optimise and as such they have yet to be generally adopted by the wheat community leaving few options for wheat breeders and geneticists who wish to carry out medium to low-density genotyping on large or very large numbers of individual plants.

The Cereal Genomics group therefore decided to investigate one of the emerging platforms further, through The University of Bristol Genomics facility which implemented a high-throughput genotyping procedure from LGC – the Kompetitive Allele-Specific

PCR (Polymerase Chain Reaction) (KASP®). They used KASP in a study to investigate the development and validation of a panel of 1114 SNPs in hexaploid bread wheat.

The KASP genotyping assay utilises a unique form of competitive allele-specific PCR that delivers highly accurate, bi-allelic scoring of SNPs and InDels at specific loci across a wide range of genomic DNA samples. KASP genotyping chemistry provides great flexibility, with the potential to support both low, mid and high throughput studies, reducing costs through eliminating the need for dual-labelled probes.

Professor Edwards explains:

“We selected LGC's KASP genotyping chemistry because it was the only platform on the market that could overcome our challenges. We had confidence in the technological ability of KASP to increase our quality of data, ensuring the accuracy and efficiency of our findings, in addition to improving the credibility and validity of our studies. We worked closely with LGC to both validate SNPs as a marker system in wheat and transfer the SNP markers, as working assays, to UK wheat breeders.”

In the study, researchers undertook to mine the UK



wheat germplasm for varietal SNPs. To do this, the group used both the available wheat expressed sequence tags (ESTs) present in the public database and next-generation sequencing (NGS), together with a novel sequence alignment and assembly approach to identify varietal SNPs in lines of interest to European wheat breeders. The group then investigated whether these SNPs could be validated by the KASP assay. The group needed a solution that would cancel out the difficulties typically associated with such technologies, creating a simpler and more efficient system for genotyping wheat.

Finally, the researchers made use of the existing Avalon x Cadenza mapping population to examine whether the validated SNPs could be efficiently placed onto the existing linkage groups identified within this doubled haploid population. For further details of the experimental procedure, results and discussion please email (genomics@lgcgroup.com) to request the application note.

Results

Using LGC's technology, the team was able to validate SNPs using KASP on 21 wheat varieties. Using the information generated from KASP, the researchers were able to identify and map 480 SNP markers to 21 linkage groups representing chromosomes, substantially more than normally categorised.

Professor Edwards found that KASP offered the flexibility and accuracy needed to handle the growing quantity and diversity of genotyping data,

“Once the study was completed, the facility functionally validated SNP assays that can be used to identify genetic markers associated with key phenotypes/traits, enabling MAS, all which will greatly improve the yield of wheat.”

Since using KASP chemistry, the University of Bristol and

the wider industry has been able to improve its practices and methodologies for providing accurate and timely data in the field of wheat genotyping. KASP has accelerated and simplified SNP genotyping for consumers, saving time and reducing costs within the industry.

Professor Edwards further explains,

“KASP increases the rate of SNP-based genotyping whilst decreasing the cost of the individual data points. With the help of KASP, our research also highlights the need to capture all of the information associated with the SNP markers, including the sequence surrounding the SNP being tested. KASP's ability to recognise all of this information enables the SNP to be adapted and used in a variety of genotyping platforms as and when they are developed. This is extremely useful, as the most productive wheat for a particular type of land can now be identified quickly and efficiently.”

Industry-wide benefits

As a result of the study, LGC, in conjunction with the University of Bristol, have developed a wheat genotyping panel, which contains over 5,000 functionally validated SNP assays that are used by researchers/plant breeders to identify genetic markers associated with key phenotypes/traits, and that enable MAS.

Each assay in the wheat panel has been designed and validated using the accurate and robust PCR-based KASP genotyping chemistry. As a result individuals can now freely obtain information about SNP markers, including the sequence upon which they are based, primers used for their identification, and haplotypes of common UK varieties. The LGC wheat genotyping panel is a useful tool for breeders, helping them to gain information on genetic relationships among individuals, vital for line and hybrid development, which is essential for increasing the yield of wheat.

Future

Increasing wheat yields is now one of the top priorities for agricultural research and the sustainable intensification of global agriculture. With the use of KASP there is now a way to increase the yield of wheat in an efficient and stable manner without compromising on food safety.

Professor Edwards comments,

“This study⁽¹⁾ was the first demonstration of KASP-based chemistry to both genotype wheat varieties and generate a linkage map. The results suggest that KASP-based genotyping is sufficiently sensitive and robust to map individual paralogous genes in the wheat genome.”

The Cereal Group at the University of Bristol in conjunction with LGC have laid a great foundation in the studies of wheat genotyping, one that the group is looking to exploit more. KASP's efficiencies can be extended to accommodate various genotyping studies, and the genomics centre at the University believe that in the not so distant future it will be possible for wheat breeders to achieve one of their most important goals, to rapidly and effectively genotype thousands of plants with a large and flexible number of markers.

Update

Since the initial study⁽¹⁾ in 2011 significant progress has been made by the team. They have developed and validated a panel of 7,228 SNPs (increased from 1,114) in hexaploid bread wheat, in addition to identifying and mapping 3,629 SNP markers (increased from 480) to 21 linkage groups representing chromosomes. If you would like to find out more and be kept informed of the latest developments register for updates: (<http://www.lgcgroup.com/genomics-info>).

(1) *Plant Biotechnol J.* 2011 Dec;9(9):1086-99. doi: 10.1111/j.1467-7652.2011.00628.x. Epub 2011 Jun 1. Transcript-

specific, singlenucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (Triticum aestivum L.), Allen AM, Barker GL, Berry ST, Coghil JA, Gwilliam R, Kirby S, Robinson P, Brenchley RC, D'Amore R, McKenzie N, Waite D, Hall A, Bevan M, Hall N, Edwards KJ. School of Biological Sciences, University of Bristol, Bristol, UK.



From left to right: Prof. Keith Edwards, Dr Sacha Allen, Dr Amanda BurrIDGE, Mr Paul Wilkinson and Dr Mark Winfield.

University of Bristol

The University of Bristol has a cluster of research and teaching excellence across the life sciences, from biotechnology to earth system science; biochemistry to experimental psychology; and individuals to societies. Specifically, the School of Biological Sciences studies everything from wheat genomics to vision ecology and animal behaviour, attracting support from worldwide funders, including BBSRC, NERC, Marie Curie and the EU.

Research activity in the School of Biological Sciences is organised into three research themes: Behaviour, Sensory and Neurobiology; Ecological and Evolutionary Processes; and Plant and Pathogen Biology. Within the Plant and Pathogen Biology department, research is being conducted into Cereal Genomics, where the aim is to explore and understand cereal genomes with a focus on wheat.

The Cereal Genomics group works closely with the University of Bristol Genomics Facility in developing resources in wheat functional genomics for use by wheat breeders and scientists. This involves wheat genotyping using single-nucleotide polymorphism (SNP) discovery and gene expression analysis using transcriptomics. The group is also investigating the organisation and function

of mismatch repair genes with the specific aim of generating new alleles for the wheat breeders. They are also heavily involved in the wheat genome sequencing project. Established in 2003, The University of Bristol Genomics Facility provides numerous services for Biological Sciences, including; next-generation sequencing, bioinformatics, microarray analysis, capillary sequencing and genotyping, real-time quantitative PCR, and quality assessment of DNA and RNA analysis. One of the University's main areas of research is looking specifically at Cereal Functional Genomics, with the aim of understanding the different varieties of wheat to enhance performance and increase productivity. Ultimately, the institution aims to provide a hub for wheat functional genomics providing UK researchers with authenticated data and biological resources, which are freely available without restriction.

Integrated tools. Accelerated science.

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