Institution: University of Bristol

Unit of Assessment: 5 – Biological Sciences

Title of case study: New genotyping tools reduce costs and improve throughput for wheat breeders, significantly advancing wheat-breeding programmes in the UK and globally

1. Summary of the impact

The wheat-breeding industry, including some of the largest plant breeders and seed-development companies in the world, has benefited from decreased production costs and increased productivity as a result of research led by the University of Bristol and carried out between 2009 and 2011. The Bristol researchers developed the tools necessary to differentiate point mutations in the complex DNA structure of wheat. This was a critical step in wheat genotyping and led to the public release of 95% of the wheat genome in 2010 and the development, by Bristol, of a cheap, easy-to-use assay for industry. These advances were quickly embraced by industrial wheat breeders aiming to deliver new varieties of wheat with improved yields and desirable traits such as disease resistance. Limagrain, the world's fifth-largest producer of field seeds (including wheat) with €595 million in sales of seeds, **realised a ten-fold reduction in costs and a ten-fold increase in throughputs in their breeding laboratory**. With the wheat-seed business worth over £16 million annually in the UK and over £1.8 billion globally, the new genotyping tools generated by Bristol have had, and continue to have, a major impact on the wheat industry and its ability to respond to the challenges of climate change and population growth.

2. Underpinning research

Key researchers and their contributions

Sequencing the wheat genome was a collaborative project between the University of Bristol, the University of Liverpool and the John Innes Centre (JIC), with Bristol being the lead organisation. Bristol generated the cDNA sequence data from the four varieties of wheat studied and used its expertise in genomics and bioinformatics to decipher the information and generate the varietal single nucleotide polymorphisms (SNPs). The team validated the SNPs in a range of germplasm to ensure their suitability for use by academic and commercial wheat breeders. This research was carried out between April 2009 and April 2011 by the following key Bristol researchers:

Professor Keith Edwards (date of appointment 1st January 2001); Chair in Cereal Functional Genomics, School of Biological Sciences
Dr Gary Barker (date of appointment February 2003); Lecturer in Bioinformatics, School of Biological Sciences

Since April 2011, Bristol has continued to work closely with the breeding companies to develop SNP markers for use in wheat breeding.

Context

SNPs are regions of DNA, between individual chromosomes, that vary by a single nucleotide. They are the most common type of genetic variation in plant and animal genomes. For this reason, SNPs are a logical choice for marker-assisted selection – a process used in breeding programmes selecting for traits such as disease resistance and productivity. However, the wheat genome is extremely complex: five times larger than the human genome, the hexaploid wheat genome has three sets of chromosomes derived from related progenitors. This has presented significant challenges in SNP discovery, as work by the Bristol group has shown that most SNPs identified represent sequence differences between the three sets of chromosomes within an individual variety rather than between chromosomes from different varieties [1]. As a result, the Bristol team had to develop and adapt a range of novel procedures in the area of next-generation sequencing, bioinformatics and genotyping in order to overcome these challenges and advance DNA marker-assisted breeding approaches.

Nature of research findings

SNP genotyping first requires the identification of valid SNPs. The Bristol team used a bioinformatics approach to electronically mine SNPs from several databases containing expressed sequence tags (ESTs) for cereals, including wheat. Their findings showed that high-throughput sequencing could generate a dataset of sufficient size to exclude SNPs within a variety from those found between varieties yet still produce sufficient SNPs to provide diverse genome coverage [2]. Simultaneously, the Bristol team was exploring high-throughput sequencing methods and developing an assay, based on novel technologies (for instance, padlock probes and Kompetitive Allele Specific PCR (KASP)), capable of simultaneously genotyping numerous wheat lines using several hundred gene-based SNP markers [3]. This work paved the way for the sequencing and genotyping of the wheat genome.

In 2010, using the wheat reference variety Chinese Spring, line 42, Bristol employed novel bioinformatics techniques to generate SNPs among the sequence information covering 95% of the wheat genome [4]. The sequence data were made available immediately in the public domain through the EMBL/GenBank and CerealsDB [5] websites, which allowed rapid uptake of this information by both the academic and commercial wheat-breeding communities.

To make the genomic sequence information accessible to industry and assist practical breeding of wheat, the Bristol team combined their bioinformatics expertise with the innovative KASP chemistry developed by the genetic-screening company, KBioscience (now LGC), to generate and validate a new type of wheat molecular marker (KASP markers) [6]. The markers are cheap and easy to use, which makes them ideal for commercial wheat-breeding programmes aimed at the fast development of new varieties selected for increased yield and resistance to abiotic and biotic stress.

3. References to the research

Outputs

[1] Barker, G.L.A. & Edwards, K.J. (2009) ‘A genome-wide analysis of single nucleotide polymorphism diversity in the world's major cereal crops’, *Plant Biotechnology Journal*, 7: 312-317. DOI: 10.1111/j.1467-7652.2009.00412.x.

[2] Edwards, K.J., *et al.* (2009) ‘Multiplex SNP-based genotyping in allohexaploid wheat using padlock probes’, *Plant Biotechnology Journal*, 7: 375-390. DOI: 10.1111/j.1467-7652.2009.00413.x.

[3] Allen, A.M., *et al.* (2011) ‘Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (*Triticum aestivum* L.)’, *Plant Biotechnology Journal,* 9: 1086-1099. DOI: 10.1111/j.1467-7652.2011.00628.x.

[4] Wilkinson, P.A., *et al.* (2012) ‘CerealsDB 2.0: an integrated resource for plant breeders and scientists’, *BMC Bioinformatics,* 13: 219. DOI: 10.1186/1471-2105-13-219.

[5] Brenchley, R., *et al.* (2012) ‘Analysis of the allohexaploid bread wheat genome using whole genome shotgun sequencing’, *Nature,* 491: 705-710. DOI: 10.1038/nature11650.

[6] Allen, A.M. *et al*. (2013) ‘Discovery and development of exome-based, co-dominant single nucleotide polymorphism markers in hexaploid wheat (*Triticum aestivum L.)*, *Plant Biotechnology Journal*, 11 (3): 279-95. DOI: 10.1111/pbi.12009.

Grants

[7] Edwards (2009-2011) *Mining the allohexaploid wheat genome for useful sequence polymorphisms*, BBSRC, £1.2 million (£250,000 to Bristol; Edwards lead PI).

[8] Edwards (2011-2013) *Development and validation of a flexible genotyping platform for wheat,* BBSRC Crop Club, £200,000 (Edwards lead PI).

Award

In 2011, Professor Edwards was awarded the Royal Agricultural Society of England’s (RASE) Research Medal for this research. The RASE Research Medal acknowledges “research work of outstanding merit carried out in the UK, which is proven or likely to be of benefit to agriculture” [a].

4. Details of the impact

In August 2010, a consortium funded by BBSRC and led by the research team at the University of Bristol released genomic sequences covering 95% of the wheat genome through the

EMBL/GenBank and CerealsDB [5] websites. This empowered wheat breeders to carry out wheat genotyping: they could begin to sequence genes of interest, such as those related to growth or disease resistance, by analysing target DNA sequences against the database supplied by the University of Bristol [b, c]. These data have been extensively accessed, with the CerealsDB website (http://www.cerealsdb.uk.net/) receiving the following numbers of visits between 2009 and 2012:

	2009	**2010**	**2011**	**2012**	**2013** (to 31/05)
Total	45,511	67,269	199,139	196,303	112,946

Evidence that the sequences available on CerealsDB have international impact can be found by examining the locations of those accessing the site's content:

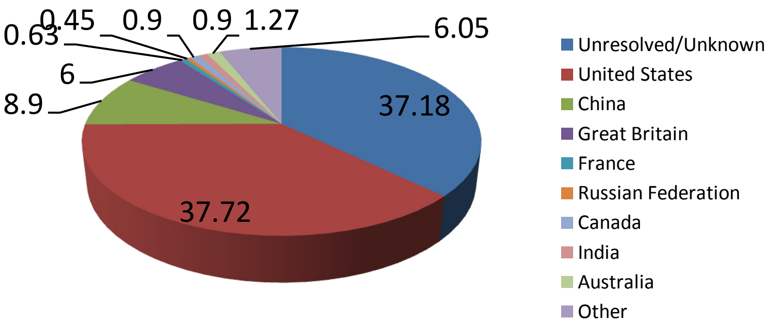

Figure 1: Summary of the locations of the users of CerealsDB (during 2012)

Research leads to new commercial genotyping service for KBioscience

For sequence information to be useful to wheat breeders, a cost-effective method of analysis and a large number of SNP markers were needed. To achieve this, the Bristol group developed novel procedures to deal with the complex DNA of wheat [2,3,4] and then collaborated with the genetic screening company, KBioscience, which had developed the KASPar assay [6].This collaboration enabled KBioscience to offer a new commercial genotyping service to the global wheat community. Though the number of screenings carried out using the wheat SNPs is commercially sensitive, the Principal Scientist at KBioscience said: "The markers are used extensively by the global wheat community and they constitute a significant part of the KBioscience portfolio" [d]. The commercial success of this new service "continues to provide benefits to the UK economy in terms of the genotyping services that KBioscience offer and via the employment of high quality technicians and scientists based on our ability to offer this service" [d].

Genotyping tools lead to decreased production costs and increased productivity for the wheat-breeding industry

The UK wheat-seed business is worth £16 million per annum, with each ton of seed having a profit value of £56-80 depending on the variety. One ton of wheat seed can plant ~7 hectares. Globally, the wheat-seed business is worth over £1.8 billion a year. The farm-gate value of wheat is £180 per ton, or £2.9 billion for the whole of the UK. The new genotyping tools generated by Bristol have had a major impact on how the wheat community carries out its genotyping, resulting in decreased costs and increased productivity for a multi-billion pound industry [b,c,e,f].

Limagrain is the largest plant breeder and seed-development company in Europe and the fifth-largest field-seed producer in the world. Field-seed sales are one of their three main activities and represent 38% of their global sales (over €1.5 billion in 2011) [g, pg. 9]. Prior to the research developments at Bristol, Limagrain was severely hampered in its objective of switching over to using SNPs, despite knowing they were the more efficient molecular marker for use in plant breeding. The research at Bristol enabled Limagrain to change from Simple Sequence Repeat (SSR) markers to SNP markers, **allowing a 10-fold reduction in the cost per data point, from €0.50 to €0.05**, due to increased automation, reduction in reagent use and improved accuracy and ease of interpretation [c]. The switch to SNP genotyping also significantly improved efficiency. The Head of Research – Cereals at Limagrain stated that "**the throughput of the Limagrain Laboratory was increased ten-fold**" as work that took three months using SSRs took just three days using the SNP markers [c].

RAGT Seeds is another one of Europe’s leading seeds businesses, with wheat being one of their key crops. The advances made by Bristol have become “embedded in [RAGT’s] systems” and are considered “**indispensable tools**” [e]. **They have led to a five-fold increase in efficiency in the RAGT laboratory**, which in turn has resulted in a doubling of the rate of genetic gain for traits such as yield, disease resistance and quality [e].

KWS, one of the leading cereal breeders in the European Union, has also benefited from this research, **realising a ten-fold increase in marker-screening capability and recently expanding their development team by two people in their cereals division** [f].

Reaching beyond the UK

All of the companies that have benefited from these advances in wheat genotyping have operations that extend beyond the UK. For example, KWS operates in 70 countries worldwide, while the Limagrain group is the fifth -largest plant breeder and seeds company in the world, employing over 6,000 people and turning over more than £1.1 billion annually.

Professor Edwards and Dr Barker and their team have consciously coordinated their research efforts with other international and UK programmes. Specifically, the SNPs identified within the project have made a significant contribution to international efforts to develop chip platforms, which offer commercial breeders an alternative assay platform for establishing large-scale patterns (F).

Significance of the impact

The wheat-breeding industry has benefited from this research since 2010, helping it to realise considerable improvements in productivity and reductions in costs. These commercial benefits have already stimulated further international investment in projects that aim to improve our knowledge and consequently our ability to exploit the genetic diversity of wheat to improve yields in the face of a growing global population and environmental change. For example, BREEDWHEAT is an international consortium of 26 partners, including industry partners such as Limagrain, with a €39 million investment programme over nine years [g, pg 23]. In 2011, BBSRC awarded a £7 million grant to a consortium of researchers, including Edwards, that aims to identify new and useful genetic traits that will improve modern wheat-breeding efforts. This collaborative work is being undertaken around the globe to try and address concerns over food security. Though considerable work remains in bringing the genomic sequencing of wheat up to the level of information that is currently available for crops such as rice, maize and soybeans, the contributions made by the researchers at Bristol represent significant advances, the impacts of which will continue to grow as new varieties of wheat are developed.

Universities and Science Minister David Willetts said that the work conducted at the University of Bristol was "… an outstanding world class contribution by the UK to the global effort to completely map the wheat genome. By using gene sequencing technology developed in the UK we now have the capability to improve the crops of the future by simply accelerating the natural breeding process to select varieties that can thrive in challenging conditions." [h]

5. Sources to corroborate the impact

[a] Royal Agricultural Society of England (2012). *What the Royal Agricultural Society of England Does – Awards.* <http://www.rase.org/what-we-do/awards/> [accessed online 4 June 2013].
[b] Head of Laboratory, Syngenta.
[c] Head of Research – Cereals, Limagrain.
[d] Principal Scientist, KBioscience.
[e] Cereal Genotyping Lead, RAGT Seeds.
[f] Barley Pre-Breeder, Cereals Division, KWS UK Ltd.
[g] *Limagrain (2011) From earth to life: Limagrain Annual Report 2011.* <http://www.limagrain.com/docs/fckeditor/file/publications/RA/Lmg_RA2011_GB.pdf>
[h] BBSRC. (2010) *UK researchers release draft sequence coverage of wheat genome.* [Press release 27 August 2010]. <http://www.bbsrc.ac.uk/news/food-security/2010/100827-pr-uk-researchers-release-draft-wheat-genome.aspx>