Small Grain Cereals

The Newsletter of UK Small Grain Cereals Research

Summer 2007 edition

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Update on SGC:

4th UK Cereals Genetics and Genomics workshop

This year's event took place in March at NIAB, Cambridge and focussed on the theme "From Genes to Traits". Many thanks to Dr. Donal O'Sullivan, Mary McPhee and Heather Barrett for organising the event! The majority of the presentations and all abstracts of this event can be accessed on the SGC website (www.smallgraincereals.org).

Exchange visits

SGC supports a limited number of laboratory visits for cereal scientists in public sector laboratories to facilitate the exchange/learning of new technologies. Visits of public sector scientists to plant breeders or other private sector laboratories for technology transfer are also supported. SGC will cover travel, accommodation and subsistence costs up to £ 500.-- and for a maximum length of 1 week. If you are interested please complete the application form on http://www.smallgraincereals.org/exchanges.htm or contact the SGC administrator by e-mail (elke.anzinger at bbsrc.ac.uk).

Monogram

Monogram is becoming established as the embodiment of BBSRCs Cross Institute activities in Grass and Cereal Genetics and Genomics. The steering group comprises Tina Barsby (Programme Manager), Andy Phillips (RRes), Graham Moore (JIC), Robbie Waugh (SCRI) and Ian King (IGER).

Monogram inherits a portfolio of BBSRC funded research in the institutes, along with SEERAD funded work at SCRI. Many of the scientists involved have a strong track record of world leading scientific

contribution. There are already many examples of projects where Monogram scientists, along with colleagues in other institutions, come together to address the major challenges facing science and society with funding from BBSRC and others. Monogram aims to further enhance these synergies, and to add value to individual areas of focus by a better alignment of resources.

BBSRC has asked Monogram to embrace relevant projects funded under the Crop Science Initiative, and the BBSRC/INRA initiative. Several of these will be familiar to the SGC community and include those led by Andy Phillips (RRes) and Margaret Boulton (JIC) 'Enhancing wheat field performance and response to abiotic stress with novel growth-regulatory alleles' and by Ian King (IGER) and John Snape (JIC) 'The establishment and application of a forward genetic resource for the development of efficient breeding strategies in grass and cereals'. These will be among the first projects featured on the Monogram website.

The development of the Monogram web site is underway, thanks to Paul Verrier at RRes who is leading on this (although individual Monogram scientists must feed in the information!). Featured projects will be outlined on the website, and it will serve as a point of contact and access to Monogram databases and resources- the intention is that this will link into a revamped Small Grain Cereals Web portal which is intended to act as a single point of contact for UK institutes and HEI resources. (Keith Edwards and Gary Barker at Bristol are leading a funding bid for this development). A message board will operate and be a useful point of contact for job adverts and details of funding opportunities. It will be especially useful for groups outside the UK to identify potential UK partners for collaborative projects which will raise the public profile of UK cereal and grass research.

A major objective for Monogram is to 'lower the threshold' to the identification and isolation of genes. Five working groups have been established (Physical mapping, Germplasm and markers, Gene expression/proteomics, Gene validation/transformation, and Bio-informatics). These will serve to encourage collaboration and sharing of resources and technical expertise. In addition, they will serve to identify and develop new resources in an integrated manner where these are required, to avoid unnecessary duplication of effort. Of course, participants from the wider SGC community are involved in these meetings and in setting the priorities for resource development and, importantly, means of access. Well organised bioinformatics resources are key. Perhaps the most urgent problem is the development of the bioinformatics network. All the institutes are committed to this and have increased the internal institute-funded staff allocated to making this happen. We are all conscious that the Brassica community is well placed to take advantage of developments in the Arabidopsis model. With Brachypodium increasingly attracting the attention of the 'model' community, monocot crop science must be ready and equipped to do the same!

Each institute has identified participating scientists and projects which fall within Monogram, and a working meeting of 23 scientists representing the core took place at Rothamsted on June 11th. This meeting was arranged in order to allow those present to meet informally, to initiate contacts with other groups, and to begin to address the practical issues that Monogram is facing, i.e. How to add value by connecting research across sites? What synergies can be identified across crop/trait areas? What are the connecting programmes? And whether the flagship programmes identified by the steering committee were appropriate. A larger meeting is planned for November 1st – 2nd 2007, details of which will be available soon. An advisory group is being formed, and is intended to be functional by this date. Peter Jack (RAGT), Andy Greenland (NIAB) and Keith Edwards (Bristol) have already agreed to bring their wheat expertise, and nominees from the other crop communities have yet to be contacted.

Importantly Monogram must also look to bring forward discoveries not only from models to crop plants, but also to delivery. This will necessarily differ from crop to crop. Some individual scientists will have individual exclusive arrangements on particular projects with breeders. At IGER, grass and oat breeding is an integral part of the Institute's activities. NIAB is active in

cereal pre-breeding. WGIN and the newly formed UK barley network (www.ukbarley.net) also have a role to play.
Monogram website: www.monogram.ac.uk

HEALTHGRAIN:

HEALTHGRAIN is a FP6 integrated project coordinated by Prof. Kaisa Poutanen at VTT, Helsinki. It comprises 43 partners from 15 countries with a total budget of about Euro 15 million. In addition over 50 companies from the food processing industries and the plant breeding sector have joined the Industrial Platform which provides workshops and networking opportunities. The aim is to improve the well being of EU consumers by increasing their intake of healthy products based on whole grains and reducing the risk of diseases related to the metabolic syndrome.

The work is divided into five modules, two of which deal with consumer research (on the expectations and acceptability of whole grain products) and with dissemination and training. The other three modules focus on plant biotechnology and crop improvement, innovative processing technologies and human nutrition.

The plant biotechnology module is led by Peter Shewry (RRes) and aims to provide sources of variation in bioactive components in cereal grain and tools to exploit them in breeding programmes.

Healthgrain field trial

Research carried out in the first two years has included an extensive "diversity screen" of 150 wheat varieties and 50 other lines of cereals which have been analysed for a wide range of bioactive compounds. Future work will include the development of a simplified "toolkit" suitable for practical use by plant breeders.

OPTIWHEAT

In July 2006 the EU funded the OPTIWHEAT project (INCO-CT-2006-015460). The project is co-ordinated by Martin Parry at Rothamsted Research and has partners in Italy (Prof. Roberto Tuberosa), Spain (Dr Jordi Bort & Prof Gustavo Slafer), Tunisia (Dr Halim Ben Haj Salah), Jordan (Dr Adnan Al-Yassin) and Morocco (Dr Mustafa Labhilili & Dr Hassan Ouabbou). In addition, Dr Miloudi Natchit, the durum wheat breeder from ICARDA, is a project advisor.
In Mediterranean countries, characterised by low and uncertain rainfall, water availability is a major determinant of crop yield. Durum wheat is one of the most widely cultivated crops. The OPTIWHEAT project seeks to both identify existing variation in Durum wheat germplasm and to generate novel genetic variation for the stability of yield under drought stress in Durum wheat.
The projects major objectives are to provide additional novel variation to Durum wheat germplasm by random chemical mutagenesis and Targeting Induced Local Lesions IN Genomes (TILLING) technology through the production of a TILLING population (Table 1).

Ethyl methyl sulphonate % (v/v)	No. Seeds treated	M2 lines giving M3 seeds
0.6	4500	2808
0.7	2000	861
0.8	2000	935

Table 1 The status of Durum wheat TILLING population in May 2007

This Durum population will complement the hexaploid and *T. monococcum* TILLING populations already in place at Rothamsted.
This population will be used for forward and reverse genetic approaches to identify lines with enhanced stable yield under drought. We will determine the novel variation for integrative morpho-

physiological traits, yield and quality within the TILLING population; 50 promising lines have already been selected by Dr Miloudi Natchit from ICARDA for further evaluation in the field. We will identify TILLING lines representing mutations in genes of interest in order to understand how the structure and expression of specific genes contribute in the context of the whole plant. Germplasm panels from other EU projects TRITIMED and IDUWUE will also be screened for variation of candidate genes in germplasm panels (ECOTILLING). This resource will be made available to other researchers and breeders to improve the sustainability of Durum wheat production under field conditions.

M3 lines growing at INRA- Settat, Morocco in April 2007

Focus on grass research: Staygreen in forage and amenity grasses

The staygreen trait, characterized by extended greenness in senescing plant leaves, is caused by a gene mutation that disrupts chlorophyll degradation. A naturally-occurring recessive staygreen mutant (sgr) in the forage grass Fetusca pratensis (meadow fescue) has been incorporated into commercial staygreen grass varieties for use in seed mixtures, where the ability to defer normal senescence produces greener landscape turf. However, the recessive character of the mutation means that traditional introgression breeding for staygreen can be a slow process. Consequently, the development of marker assisted selection protocols became a priority.

Gene identification: a mapping family containing the Festuca-derived sgr on a single introgression in the Lolium multiflorum (Italian ryegrass) background was established and this introgression was mapped to Lolium/Festuca chromosome 5 (C5). Previous comparative genetic studies had established a syntenic relationship between this region of C5 and a region of rice C9 which also contained a staygreen locus. Fine mapping in Lolium/Festuca indicated that sgr was likely to be one of 30 gene models (based on synteny) on rice C9. Existing annotations of these gene models in the rice genome identified LOC_Os09g36200, a senescence-inducible chloroplast stay-green protein, as a promising candidate for sgr.

Gene validation: to validate the function of the sgr candidate gene, 2 approaches were taken:
A) Temporal and organ-specific expression patterns of Arabidopsis genes orthologous to all the 30 candidate rice genes in the fine-mapped syntenic region were analyzed using microarray data available in the Genevestigator® Meta-Analyzer database. These data identified At4g22920 (the most similar Arabidopsis protein to LOC_ Os09g36200) to be clearly up-regulated for expression during the period of maximal senescence in leaves in Arabidopsis, placing gene activity in the right timeframe and tissue for involvement in leaf senescence.
B) RNA interference (RNAi) was used to silence the candidate sgr gene in Arabidopsis (At4g22920) resulting in plants with a staygreen phenotype equivalent to the original sgr *F. pratensis* plant. Chlorophyll degradation in the Arabidopsis RNAi lines was greatly reduced compared to control plants after dark incubation consistent with a role for sgr in chlorophyll catabolism.
The results from these two approaches strongly suggested that the orthologous gene in *F. pratensis* is responsible for the staygreen trait.

Marker development: the full-length Lolium orthologue of rice LOC_ Os09g36200 was obtained by screening a Lolium BAC library and this sequence was used to develop PCR primers to amplify regions of the sgr candidate gene from staygreen ryegrass lines. A four base-pair insertion was identified in the second predicted exon of the candidate mutant (sgr) gene. This frameshift insertion is predicted to dramatically

change the wild-type (SGR) protein sequence after amino acid residue 100, including truncation to 232 residues in sgr from 279 in SGR. This 4 bp insertion forms the basis of a molecular marker which co-segregates 100% with the staygreen trait in both mapping and breeding populations of Lolium/Festuca.

Full details can be found in: New Phytologist, 172:4, 592-597 (2006), Science, 315:5808, 73 (2007).

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UK Barley Network

The UK Barley network was launched on the 11th of June and aims to include all UK barley research. The founder members are SCRI, CPB Twyfords, Syngenta, New Farm Crops, Nickerson, Advanta, Lemke Serasem, Plant Breeding and Secobra/Dalgety.

Mission:
To co-ordinate and promote pre-competitive barley research in the UK and to communicate the anticipated benefits to the end-user community.
What is its function? The UK Barley Network will serve as a single point of contact in order to:
1. Consolidate UK applied barley.
2. Enable innovation at fundamental and applied levels to be linked to the delivery of more competitive products in national and international markets.
3. Communicate research requirements in a two-way dialogue with policy makers and research funders.
4. Improve education and training in plant breeding and applied barley genetics.
5. Provide an information exchange point for UK barley.

What is NOT its function? UK Barley Network will not act directly to:
1. Develop its own research proposals.
2. Develop its own intellectual property or that of a member.

Website: www.ukbarley.net (This website will be up and running by the end of July.)
Contacts:
Bill Thomas: Bill.Thomas@scri.ac.uk
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Two new barley ERA-PG projects funded!

Genomics-assisted dissection of barley morphology and development (BARCODE)

Since the middle of the last century barley has been an established model for plant genetic research. Latterly, its small but focussed research community has fully embraced the concept of global cooperation and articulated a single genomics vision and roadmap towards an ultimate objective of obtaining a full genome sequence. The highest priority in the barley genomics vision is now the development of a genetically anchored physical map of the genome, to provide a platform for both forward genetics and, in the future, targeted and whole genome sequencing projects. The key impact of the physical map will be to enable the genetics community to address important biology-driven questions rtant crop plant.

So how will the transition from focussing on resource generation to working on biological targets be achieved? If, as BARCODE proposes, the research trajectory in barley parallels that followed by the Arabidopsis community, then the ability to knock out, or change a specific gene function or trait phenotype by biologically, physically or chemically inducing mutant alleles will feature highly. Such induced mutations themselves have, in most cases, no commercial or

agronomic value (although there are many that do). However they frequently generate phenotypes that can be exploited directly to identify and isolate the disrupted genes. Knowing how these extreme or deleterious lesions in a specific gene affect a phenotype provides an opportunity to address fundamental mechanistic and regulatory questions but also to embark upon sequence based natural-diversity analyses and the possibility of applying variant alleles in crop improvement. Induced mutations were first reported in Drosophila in 1927 by H. J. Müller and over the next 70 years, barley, a true diploid inbreeding species, emerged as the model plant for testing physical and chemical agents for their mutagenic effects. During this period, a large body of work resulted in the accumulation of extensive and well characterised mutant collections in many laboratories. However, as interest in these collections peaked in the 1960s, with the exception of a few examples (e.g. Hooded), none have been systematically exploited to address focussed biological questions using the tools of modern genetics. BARCODE proposes that given current advances in genomic technologies it is time for this position to change.

In BARCODE, the three collaborating groups (Robbie Waugh, SCRI, UK, Nils Stein IPK, Germany, and Michele Morgante, Udine, Italy) will map ~ 1000 mutant alleles that are present in a collection known as the 'Bowman nearly isogenic lines'(Bowman NILs) which have been developed by Prof. Jerry Franckowiak over the last 20 years. They represent a significant portion of the viable, visual deviants reported in barley (described in the Barley Genetics Newsletter Vol 26 (http://wheat.pw.usda.gov/ggpages/bgn/) with pictures available at http://ace.untamo.net/bgs/.). They are an immensely powerful yet largely untapped resource for investigating unique morphological and developmental questions in *Triticeae* biology. The relative position of each mutant locus on the barley genetic map will be experimentally determined and then translated through linked gene sequences to discrete physical locations on the barley physical map. The latter will be achieved by determining the paired-end sequences of ~400K Barley BAC clones, incorporating that information into ongoing efforts to generate a BAC-based physical map and exploiting conserved synteny with sequenced model genomes. During the project the participants will conduct a series of proof of principle experiments aimed at cloning several of the morphological mutant loci.

Genomics-Assisted Analysis and Exploitation of Barley Diversity (EXBARDIV)

Crop plants have evolved from their wild ancestors during domestication and selective breeding over the last *ca.* 10 000 years. First, wild plants carrying promising traits were cultivated, leading eventually to locally adapted landraces. Modern breeding has taken a limited selection of this germplasm and crossed the 'best with the best' to yield modern cultivars. Unfortunately, there are indications that we are approaching a performance ceiling for at least some crops, as the best alleles available become combined together. A potential escape route from this cul-de-sac is provided by introducing fresh alleles into cultivated materials from wild and old, locally adapted germplasm. This approach has already been successful in introducing resistance genes from the wild into cultivars. The challenge for future molecular breeding is to streamline this process, using high throughput genomics approaches, to handle less tractable but equally important traits affecting yield and adaptability.

One particularly promising approach is association analysis, which compares genotype and phenotype data for heterogeneous populations and looks for links between these two parameters. This approach is intrinsically more powerful than 'classical' genetic linkage mapping because it scrutinises the results of thousands of generations of recombination and selection. However, association mapping faces a paradox - it is relatively easy to detect marker-trait associations in highly inbred populations, such as modern cultivars, but this inevitably results in a low resolution map, requiring more work to pin down the gene allele responsible for the trait. Conversely, highly diverse populations provide high-resolution associations but the numbers of markers needed to find any association are extremely high. Nevertheless, association mapping has been fruitful in human

genetic studies and is just beginning to be tested in plants. Barley is an ideal prototype for such a study, largely because thousands of gene-based SNP polymorphisms are available. The goal of the EXBARDIV project is to apply these high throughput markers to European barley cultivars and combine these data with detailed phenotypic analysis to identify new associations. These promising 'low resolution' associations will be refined to yield more accurate associations by repeating the study in locally adapted barley landraces and, if necessary, a final high resolution association analysis will be performed in a wild barley collection to pin down the exact gene alleles responsible for the traits.

The EXBARDIV project brings together 7 European research institutions with extensive experience in barley genome and phenotype analysis. The University of Dundee (Andy Flavell, coordinator) and the Scottish Crop Research Institute (Joanne Russell, Robbie Waugh) represent the UK, the Max Planck Institute (Klaus Pillen, MPIZ-Cologne) and the Institute of Plant Genetics and Crop Plant Research (Andreas Graner, IPK-Gatersleben) represent Germany and Italy, Finland and Denmark are represented by the Experimental Institute for Cereal Research, Fiorenzuola d'Arda (CRA, Luigi Cattivelli), MTT Agrifood Research (Alan Schulman) and the University of Copenhagen (Søren Rasmussen) respectively.

TritiGen COST action FA0604:

This Europe wide initiative has now been funded through till June 2011, and details are available from: http://www.cost.esf.org The main objective of the programme is "The development of technology platforms and coordinating projects to identify and exploit qualitative and QTL alleles for improving wheat barley and rye". Arranged along similar lines to Monogram, with four working groups underpinned by Bio-informatics, the UK representatives on the management committee are Tina Barsby and Robbie Waugh. A number of UK research groups are represented, and the next meeting, where there will be a series of workshops, is in October 2007 in Tenerife immediately prior to the PlantGEMS 6 meeting (http://www.plant-gems.org). Anyone can attend these. There is funding for two UK scientists per working group to attend meetings as "eligible participants". Eligible participants need to be actively participating in the meeting (e.g. giving a talk, poster etc) and need to register on the COST web site. The European Triticeae Genomics Initiative workshop will also take place at this meeting on 5th October 2007, and there will be discussions about the new EU Framework funding. An ERA-PG meeting is planned also. It is important that we encourage UK scientists to attend and to participate actively in these projects – so mark your diaries.

Please contact Tina (tina.barsby@niab.com or tina.barsby@bbsrc.ac.uk) or Robbie (robbie.waugh@scri.ac.uk) for further information. They are aiming to co-ordinate participation and to keep a running list of attendees, including those eligible for funding. We would like to encourage young and less well-established scientists by allocating the funding to them.

Many thanks to Ian Armstead (IGER), Ian King (IGER), Martin Parry (RRes), Robbie Waugh (SCRI), Tina Barsby (Monogram), Peter Shewry (RRes) and Bill Thomas (SCRI) for contributing to this newsletter. This newsletter was edited by Elke Anzinger.
Web: http://www.smallgraincereals.org