Next WGIN stakeholder meeting – 29th November at Rothamsted Research

Defra Wheat Genetic Improvement Network – The Core Research Project

Background
The UK government is committed to a more sustainable agriculture. Wheat is grown on a larger area and is more valuable than any other arable crop in the UK. The overall aim of this project is to generate pre-breeding material carrying novel traits to the UK breeding companies and to deliver accessible technologies thereby ensuring the means are available to produce new, improved varieties. An integrated scientific 'core' which combines underpinning molecular markers, genetic and genomic research, together with novel trait identification, are being pursued to achieve this goal. The programme is managed by a team including representatives of the key UK research groups and breeders. They ensure the programme and its outputs are communicated to the wider scientific and end user communities, via a web site, a stakeholder forum, focused meetings and peer reviewed publications. The WGIN will ensure collaborations with equivalent operations overseas to ensure the programme is internationally competitive.

This project
The Core Project started in 2003 provides genetic and molecular resources for research in the Satellite Defra Projects and for a wide range of wheat research projects in the UK. These resources include wheat genetic stocks, mapping populations, molecular markers and marker technologies, trait identification and evaluation, genomics and bioinformatics. The Research Platform will promote the integration of the funded work.

UPDATE ON PROJECT OBJECTIVES - FOCUS ON PHENOTYPES

Focus on phenotypes at the John Innes Centre (Objectives 2 and 7)

The search for new alleles and allelic combinations with benefits for breeding and agriculture is what WGIN is all about. To find these alleles we need detailed phenotypic description of our germplasm, which, when coupled to genotypic information provides a great community platform for dissection of key traits. At the John Innes Centre (JIC) the germplasm developed as a WGIN resource includes:

The AE Watkins collection - more than 800 diverse land races of wheat collected in the early 20th century from around the world. This is a truly unique collection of alleles and allelic combinations of genes that modern breeders may have left behind.

Paragon mutant populations - 7000 individual lines derived by multiple rounds of single seed descent (SSD) from a population of Paragon treated with EMS, a chemical that causes tiny genetic changes called mutations. Can we identify new useful alleles? Or use induced variation in known genes to better understand how they work?

Avalon x Cadenza double haploids - The majority of the important variation that breeders have to deal with is quantitative, traits differing between two parents are not inherited simply when you cross those varieties. The role of this WGIN population is to reveal which parts of

different wheat chromosome contain genes that when shuffled in different combinations account for all the variation seen in the 206 children of the Avalon X Cadenza family!

In order to exploit these germplasm resources scientists and breeders need phenotypic data. Most of the germplasm described has been analysed for height, flowering time, yield, senescence, grain shape, grain size, and spike characteristics. Details of phenotypic and genotypic data can be found on the WGIN website (http://www.wgin.org.uk/).

Next Steps in Phenotype Dissection

The next stage of this work is more detailed phenotypic dissection of traits and association with genotypic variation. For example lines of the AE Watkins collection show spring and winter growth habits (Figure 1).

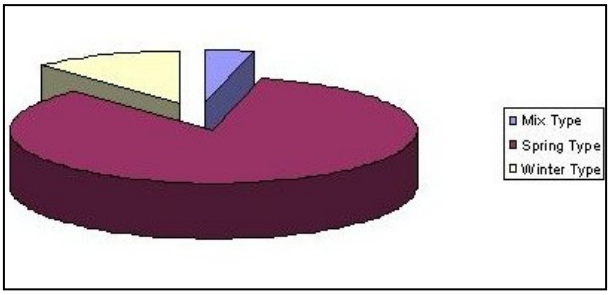


Figure 1: Vernalisation requirements of the AE Watkins collection

The winter types also appear to display variation in how long a cold treatment they need (vernalisation requirement) before they can make the physiological transition from making leaves to making spikes. Using markers developed to reveal the mutations that change winter wheat into spring wheat (Fu et al., 2005), Leodie Alibert of JIC is showing which alleles are present in the Watkins collection (Figure 2). If the variation in growth habit is not explained by known alleles then key genes will be re-sequenced to show what mutations are responsible for the observed phenotype.

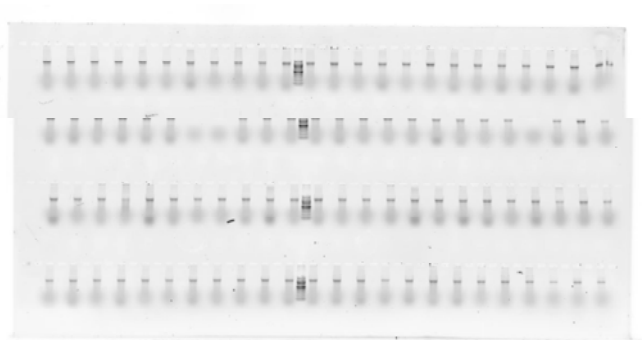


Figure 2: Checking the presence of the VrnA1 winter allele in a subset of the AE Watkins collection

Our in depth analysis of Paragon mutants takes the form of genetic mapping carried out by Simon Orford of JIC. A chromosomal location shows if the mutant locus is likely to be a new gene or an allele of something already described. Some of the phenotypes selected for further characterisation are shown in Figure 3. In Figure 4 the segregation of a gene influencing plant height is shown.

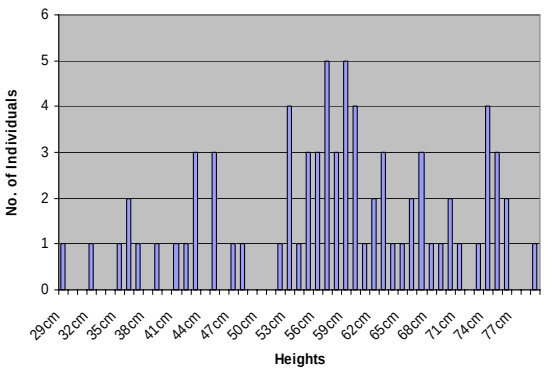


Figure 4: F_2 Distribution of plant heights of Paragon mutant 2566b x Cadenza

Figure 3: Paragon mutant phenotypes. Late and early senescence and variation in height.

This data will allow this mutation to be genetically mapped. Mutant lines from this population have been taken on for further analysis at laboratories around the world. For example scientists at NIAB (Cambridge, UK) are identifying Paragon mutants that produce low levels of Phytic acid, CIMMYT (Mexico) are studying differential response to drought, INRA (France) use our low tillering mutants to understand tiller initiation, BBSRC-INRA workers are studying Nitrogen Use efficiency, and numerous breeders are screening this unique population.

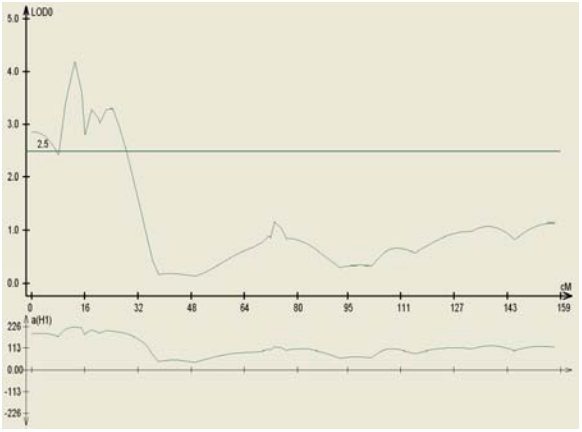


Figure 5: The large peak represents a QTL for yield on the short arm of chromosome 3B. The allele from Avalon is responsible for a 0.2 tonne per hectare increase in yield.

Phenotyping of the Avalon x Cadenza population is carried out at JICs Church Farm field trial site by Liz Sayers. Year on year measurement of the same set of key traits not only allows the identification of the QTL controlling them, but the changing penetration of these QTL from year to year is compared to detailed weather records, site, and disease records. A key question for breeding and gene discovery is why any particular allele is effective in one environment but not another. A QTL for yield, the most important but also most environmentally sensitive trait, is shown in Figure 5.

Reference: Fu, D.L., P. Szucs, L.L. Yan, M. Helguera, J.S. Skinner, J. von Zitzewitz, P.M. Hayes, and J. Dubcovsky. 2005. Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat (vol 273, pg 54, 2005). Molecular Genetics and Genomics 274:442-443.

Exploiting *Triticum monococcum* as a model for detection of traits, genes and variant alleles and for identifying phenotype: genotype relationships (RRes) (Objective 6)

Wheat genetic improvement relies on the enlargement of gene pools by introducing novel traits from closely-related germplasm. *T. monococcum* is a diploid einkorn wheat ($2n=2x=14$, A^mA^m genome) closely related to the hexaploid wheat. Although domesticated 8000 years ago and dominating early human farming activity, *T. monococcum* has been infrequently used in wheat breeding. This unique history of *T. monococcum* coupled with the availability of *ex situ* and *in situ* collections of many land races makes this species potentially useful for modern wheat genetic improvement. *T. monococcum* was explicitly introduced into the WGIN core project for the identification and exploitation of new traits. Here we report our recent progress in characterising resistance to three major pathogens of UK wheat crops.

Septoria tritici leaf blotch

Septoria tritici leaf blotch disease is caused by *Mycosphaerella graminicola*. This is the UK's number 1 wheat disease. In the April 2006 WGIN Newsletter we reported the discovery of a high level of resistance to Septoria tritici leaf blotch in *T. monococcum*. This is manifested as the complete inhibition of the formation of necrotic lesions and asexual pycnidial spores (Figure 6).

Figure 6: High level of resistance to Septoria tritici leaf blotch is found in T. monococcum. Shown are the severe disease symptoms in the field plots of hexaploid wheat variety Hereward, whereas no disease lesions were found in T. monococcum. This result has been confirmed in 4-years of field trials at Rothamsted Research.

We have continued field assessments for four consecutive years at multiple sites and the results indicate that the resistance is still 100% effective. This resistance to *M. graminicola* was found in all the accessions tested suggesting that

it is a common trait in *T. monococcum*. Thus, *T. monococcum* is a good source of resistance to *M. graminicola*. So far, one locus conferring resistance to two different races of *M. graminicola* has been mapped to chromosome $7A^m$ in *T. monococcum*.

Cereal eyespot

Cereal eyespot is a stem-based disease caused by two closely related fungal species *Oculimacula* (=*Tapesia*) *yallundae* and *O. acuformis*. Infection results in severe weakening of wheat straws and subsequent crop lodging. To assess the potential resistance of *T. monococcum* to eyespot, replicated field trials were carried out for three years on the Rothamsted Research farm. In addition to naturally occurring inoculums, the disease pressure was increased by adding artificial inoculums of both *O. yallundae* and *O. acuformis*.

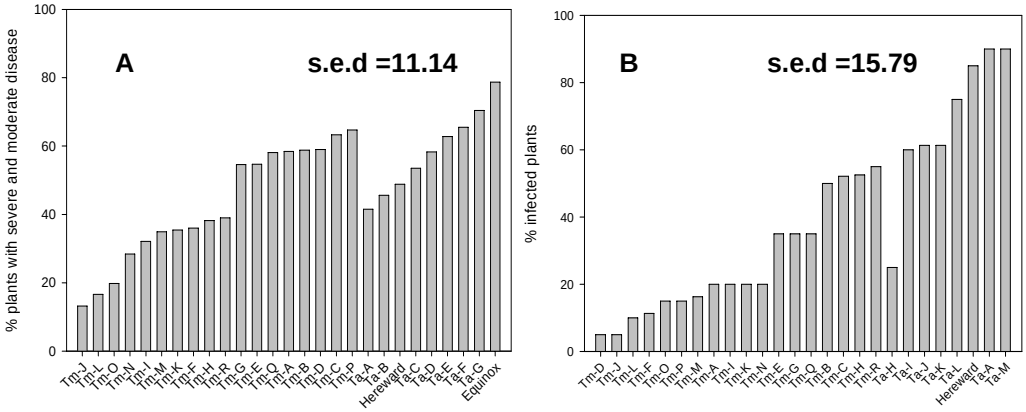
Figure 7: Eyespot resistance in T. monococcum. The left panel shows eyespot infected stems of susceptible hexaploid wheat. The middle and right panels show respectively a susceptible and a resistant T. monococcum genotype

The artificial inoculums were in the form of colonised oat grains and were applied in November or December at growth stage 12. High level of infection occurred in susceptible hexaploid wheats and some *T. monococcum* accessions, but a high level of resistance was also found in *T. monococcum* (Figure 7). Compared to the hexaploid wheat varieties examined, some *T. monococcum* accessions exhibited higher resistance levels (Figure 8, left).

To further validate the resistance observed in field trials, pot assays were carried out under controlled environmental conditions, which were adjusted to favour the growth and infection of eyespot fungi and created higher disease pressure. The infection was done by attaching ring-shaped agar plugs containing a mixture of *O. yallundae* and *O. acuformis* onto the stems of two-week-old seedlings and the disease symptoms were assessed 12 weeks after inoculation. As shown in Figure 8 (right), the accessions with high level of resistance in the field trials consistently exhibited lowest disease symptoms in the pot assay, but some accessions (e.g. Tm-D and Tm-P) with high resistance in the pot assay showed high disease under field conditions. Some accessions had less than 10% infected plants, much lower than that of the most resistant hexaploid wheat variety (e.g. Ta-H). Thus, both field and controlled environment tests demonstrate that *T. monococcum* is a good source of resistance to eyespot.

We are currently interacting with Professor Tim Murray's group at Washington University, USA to quantify individually the biomass of *O. yallundae* and *O. acuformis* in the best *T. monococcum* genotypes identified in this study. This will be assessed by using individual eyespot reporter strains which constitutively express the *GUS* gene (Cadle MM, Murray TD, Jones SS, 1997, Plant Disease 81, 1181-1186.). The same genotypes will also be assessed for their resistance to the UK eyespot species by RAGT.

Figure 8 below: Comparison of eyespot disease symptoms in hexaploid and diploid wheat. Shown are results from a field trial (left) and from a controlled environmental pot assay (right). A standard disease scoring system was used (Scott and Hollins, 1974, Annals of Applied Biology 78, 269-279.).



Take-all disease

Take-all, caused by *Gaeumannomyces graminis* var *tritici*, is one of the most damaging diseases of wheat and barley in UK. It is considered the primary cause of the 'second wheat syndrome' and hence restricts cereal rotational options. It is a soil-borne fungus which attacks cereal roots. In severely infected fields the disease, which often occurs in patches, causes stunted plants, premature senescence, white heads and a severe loss of yield (Figure 9). Global research in the past thirty years has failed to identify any good sources of resistance in hexaploid wheat germplasm. To our surprise, in both field and controlled environment experiments, large variation in the development of take-all disease symptoms was found amongst *T. monococcum* accessions (Figure 10). Some *T. monococcum* accessions exhibited a similar level of susceptibility to take-all as hexaploid wheat varieties. In contrast, some *T. monococcum* accessions had much low levels of diseases. Several accessions consistently showed low levels of diseases in both three-years of field trials and replicated pot experiments.

A preliminary experiment done at Rothamsted Research in 2007 has confirmed that cereal species differ in their overall susceptibility to take-all; wheat is the most susceptible, rye the least and barley and triticale intermediate (Getteridge RJ, Hornby D, Hollins TW, Prew RD, 1993, Plant Pathology **42**, 425-431.). Different cereal species will be incorporated into the 2008 field experiment to compare with the most promising *T. monococcum* accessions.

In summary, our intensive screen carried out over 4 years indicates that *T. monococcum* is a rich source of resistance to major UK wheat pathogens. Introgression of the identified novel traits may allow for breeding of new wheat varieties with enhanced resistance. Currently we are developing various mapping populations and a high density molecular marker map to pinpoint the genetic loci conferring the novel resistance traits. The markers closely linked to the genetic loci will be used for efficient breeding selection. In the longer term, our aim is to identify the molecular nature of the genetic loci. This new knowledge should provide a mechanistic understanding of resistance to non-biotrophic fungi as well as permit the development of 'within-the-gene' markers for efficient molecular breeding.

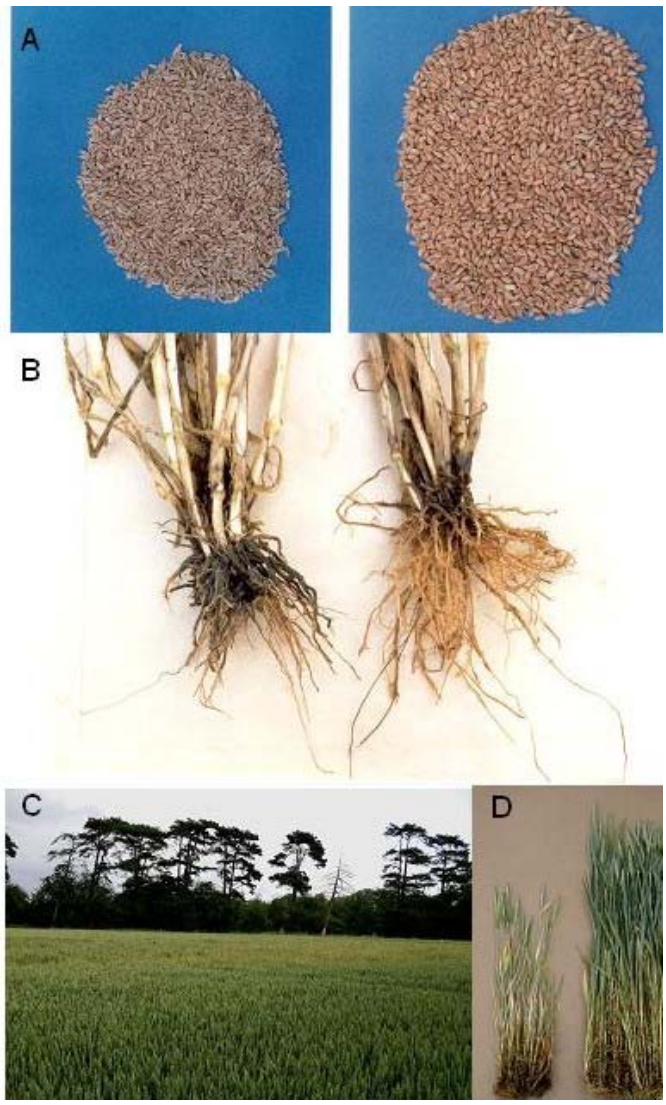


Figure 9: Take-all root rot can be a big threat to UK wheat production. (A) Grain harvested from a take-all infected (left) and a non-infected (right) field. (B). Root systems of infected (left) and non-infected (right) wheat plants. The infected plants have black roots. (C) A Rothamsted Research field with severe disease symptoms. The top part of the graph was the infected area. (D) Plants from the infected (left) non-infected (right) area. Photos C and D were taken in June 2007.

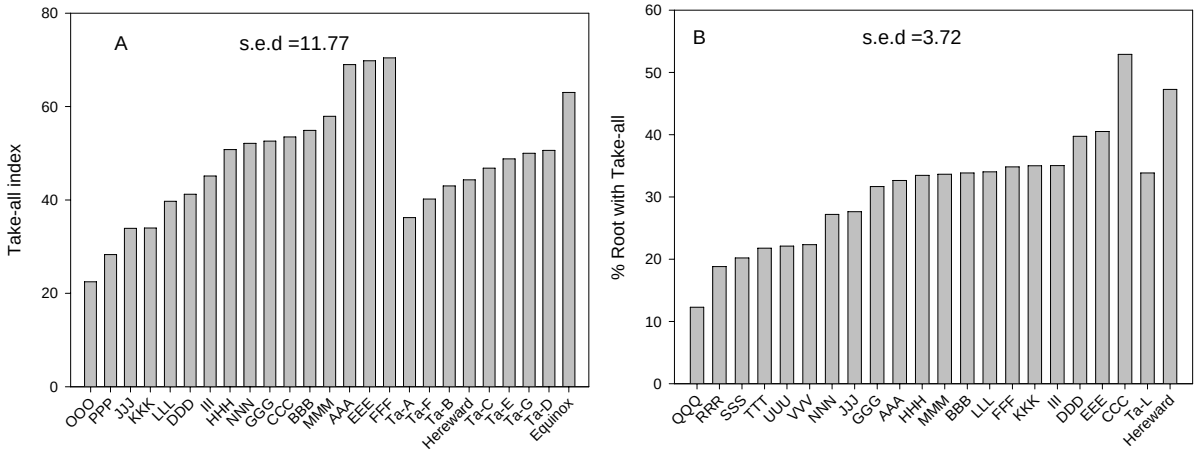


Figure 10: Comparison of take-all disease symptoms in hexaploid and diploid wheat. Shown are results from a field trial (left) and from a controlled environmental pot assay (right). Note that T. monococcum accessions used for field and pot experiments were not totally identical. The take-all index definition and calculation was adopted from Bateman et al (Bateman GL, Gutteridge RJ, Jenkyn JF, 2004, Annals of Applied Biology 145, 317-330) The hexaploid wheat experiments are funded from other sources and are included for comparative purposes.

Full details of the *T. monococcum* accession collection used in these phytopathology experiments will soon be published in the Journal of Experimental Biology.

The Cadenza mutagenised population – a resource for forward and reverse genetics. (RRes) (Objective 9)

The mutagenised population of bread wheat developed at RRes comprises approximately 4,200 independent lines, derived from seeds of the spring cultivar Cadenza treated with the mutagen ethyl methanesulphonate. The population was originally intended as a resource purely for reverse genetics, using the TILLING protocol developed at the Fred Hutchinson Cancer Research Center in Seattle to identify mutants at the DNA level. The TILLING protocol has been successfully established at RRes, supported by WGIN and by a grant from the BBSRC Research Equipment Initiative, although further development work is required to improve the robustness of the technique.

A TILLING screen for mutations in the GA20ox1 gene of wheat, which is implicated in the control of both dormancy and plant architecture, indicated that the frequency of mutations in this population was in the region of one point mutation for every 20,000 base pairs. At this mutation rate it will in principle be possible to identify a wide range of potential knock-down and null alleles for most gene targets; indeed, probable null mutations in both A and B homoeologues of GA20ox1 have been identified, which will allow the role of this gene in key architecture and quality traits to be investigated.

Despite the hexaploid nature of bread wheat, which permits a high mutation rate due to complementation of most individual mutations by the homoeologous copies of the genes, the population also has a high rate of observable phenotypes. In collaboration with the Plant Breeding and Biotechnology module, led by Prof. Peter Shewry, of the Sixth Framework Programme “Healthgrain”, the population has been phenotyped in the field at the M5 generation, at Martonvásár, Hungary. We identified mutations in a range of traits such as flowering time, plant height, virus resistance, fertility and ear morphology such that 25% of the lines were affected in some character. This suggests that this population could represent a valuable source of variation in key traits.

In addition to phenotyping in the field, we have collaborated with other groups to identify mutations in specific traits and pathways. For example, Francesco Sestilli and Domenico Lafiandra of the University of Tuscia, Viterbo, have screened for mutations in starch biosynthesis using SDS-PAGE gels of starch granule proteins. Using this method, the three homoeologous SGP-1 proteins can be resolved and loss of individual isozymes identified. Screening 500 EMS lines of Cadenza yielded

eleven candidate mutant lines each showing loss of one of the homoeologue-specific polypeptides (Figure 11).

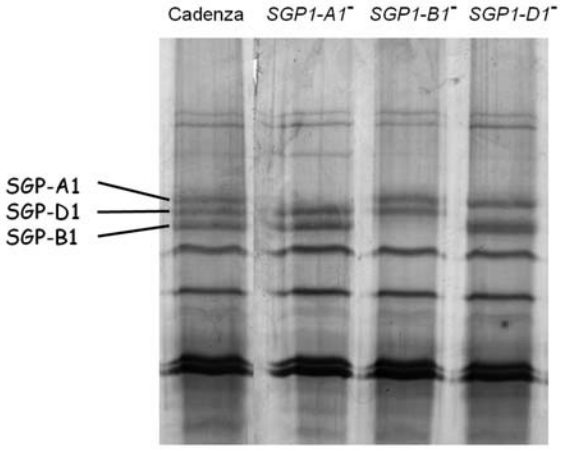


Figure 11: Identification of mutants in starch biosynthesis using SDS-PAGE to resolve isozymes of starch granule protein-1 (SGP-1).

Also under the Healthgrain project, Mariann Rakszegi of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, has screened the population for mutations affecting pericarp colour and identified four white mutants, candidates for lesions in the single functional *R* (Red Pericarp) gene that is present in Cadenza (Figure 12).

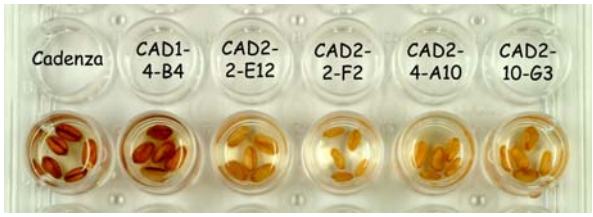


Figure 12: White pericarp mutants identified by NaOH treatment of seeds from the EMS population. Five potential lines were identified at M_3, of which four were confirmed at M_4 as shown.

The EMS-mutagenised Cadenza population is available on request, although a handling charge may be levied to allow for packaging of the four thousand-plus lines. Groups wishing to collaborate on this or in TILLING for candidate genes should contact Andy Phillips, RRes (andy.phillips@bbsrc.ac.uk).

For further information on the WGIN project please see www.wgin.org.uk or contact us at wgin.defra@bbsrc.ac.uk

The contributors to this newsletter were: At Rothamsted Research: Andy Phillips, Hai-Chun Jing, Richard Gutteridge and Kim Hammond-Kosack. At the John Innes Centre: Simon Griffiths, Simon Orford, Leodie Alibert and Liz Sayers.

Next WGIN stakeholder meeting: 29th November 2007 at Rothamsted Research

This year's topics are:
Introduction to WGIN – Kim Hammond-Kosack (RRes)
The WGIN Diversity trial – Malcolm Hawkesford (RRes)
Developing a Mutant Wheat Resource – Simon Orford (JIC)
Take All Resistance – Richard Gutteridge (RRes)
Changes in the Wheat market:

- Biofuels – Richard Weightman (ADAS)
- Climate change – David Lawlor (RRes)