Wheat Genetic Improvement Network

Special WGIN Resources Newsletter

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

A.E. Watkins Collection (JIC)
Objective 2

The A E Watkins wheat collection, which is held in the John Innes Centre germplasm collection, was collected in the 1930s, using connections Watkins (at the University of Cambridge) had with the London Board of Trade. Wheat landraces and varieties were collected from markets from 32 countries across the world. In total, 814 lines of the collection have been studied for phenotype variation. Heading time, height and vernalisation requirements were determined in the 2006 Church Farm Bawburgh field trial and this was used to ascertain the uniformity within individual accessions.

Due to the nature of the sampling of the original material, from local markets, it would be expected that the material would be heterogeneous within accessions. To assess the degree of uniformity after many years of selfing the original accessions, four individual seeds were taken from each accession and grown under glass. One metre rows were then grown in the field trials from bagged seed of each of these four sub samples.

Figure 1: Watkins collection at John Innes Centre, Church Farm

A great diversity between accessions was evident from phenotype scores. Heading time varied from 77 – 120 days and heights of 50 – 150 cm were recorded. Due to the spring sowing, 12% of lines showed some evidence of a vernalisation requirement. Also 28% of the Watkins Collection tested in the field showed signs of heterogeneity for at least one of the three phenotypes used in the assessment. Seed and DNA stocks of all the new sub samples have been stored along with specimen ears. With the segregating accessions, the collection has the potential to now increase in size. Visit www.wgin.org and follow links to Watkins Collection Sort Sheet or contact mike.ambrose, simon.orford or simon.griffiths all @bbsrc.ac.uk for resource requests – seed and DNA or further information.

Wheat cultivars used in the WGIN Nitrogen Use Efficiency trials: Objective 5 Years 1 – 5 (RRes)

For each of the cultivars listed overleaf (Table 1) and for each year indicated a 1 kg grain sample has been dried to 12% moisture content and then stored in a sealed box at -20°C. For each cultivar all the experimental replicates for each N regime used are available. Small grain samples can be requested from the seed archive at Rothamsted Research for any experimental purpose via the WGIN e-mail address.

For the field experiments harvested in 2006 and 2007 additional samples (ranging from 1 – 10 kg) have been stored at room temperature from each N regime / genotype / replica. Again grain samples can be requested for experimental purposes. The genotypes to be included in the 2008 trial are also indicated. Those only sown in the 2004 trial are listed at the foot of the table.

The double haploid Avalon x Cadenza mapping population (n = 203 lines) along with the two parental lines are being grown in replicated trials at a single N rate at both the Rothamsted Research and Woburn sites in the 2006/2007 season. Soil cores were taken in early March to assess residual soil N. As a minimum we plan to measure plant heights and final grain yields. In addition 1 kg grain samples from each plot will be archived. Some lines will be selected for N measurements in the grain and straw. The experiment will be repeated at both sites in the 2007/2008 season.

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

To achieve WGIN Objective 6, many novel genetic resources and molecular tools have been developed for the diploid einkorn wheat *Triticum monococcum* (2n=2x=14, A^mA^m).

1. A global collection of *T. monococcum* accessions has been assembled at Rothamsted Research

The collection consists of 246 *T. monococcum* accessions (Table 2). The germplasm was obtained from various sources including the Vavilov Institute (VIR) in Russia (24 landraces), IPK-Genebank in German (139 accessions), USDA GRIN in the USA (78 accessions), John Innes Centre (3 accessions), Prof. Francesco Salamini (1 accession) at The Max Planck Institute of Plant Breeding at Cologne, Germany and Prof. Jorge Dubcovsky at the University of California at Davis, USA (1 accession). The 24

Origin Country	Numbers	Origin Country	Numbers	Variety	Numbers	Other features	Numbers
Algeria	1	Ukraine	2	MDR050	1	Seasonality	
Chechen	1	Armenia	3	DV 92	1	Spring	207
Czechoslovakia	1	Austria	3	*PI355520*	1	Winter	35
Denmark	1	Georgia	3	L118	1	Facultative	1
French	1	United States	3	*kaploutras*	1	Intermediate	1
Iran	1	Germany	4	*kelcyras*	1		
Israel	1	Romania	4	*mansfeldii*	1	Earliest collection time	(Year 1904)
Kenya	1	unknown	7	*viridivulgare*	1		
Russian	1	Yugoslavia	7	*laetissimum*	2	BAC library	1
South Africa	1	Balkans region	8	*sofianum*	3	(MDR308/DV92)	
Syria	1	Greece	9	*atriaristatum*	5		
Azerbaijan	2	Italy	9	*hohensteinii*	6	Transformable	2
Ethiopia	2	Spain	9	*nigricultum*	6	accessions (MDR001	
Hungary	2	Bulgaria	11	*monococcum*	9	EMS populations	2
Iraq	2	Europe	39	*flavescens*	13	(MDR050 and MDR308)	
Morocco	2	Albania	45	*hornemannii*	21		
Sweden	2	Turkey	55	*macedonicum*	28	Ion beam irradation	1
Swiss	2	Total	246	*vulgare*	66	populations (MDR308)	
lands				*unknown*	79		

Table 2. Features of the Triticum monococcum collection at Rothamsted Research, UK

Variety	Data (04/05/06/07/08)	Nabim	Rationale
1. Avalon	No/05/06/07/08	1	WGIN DH parent; Low NupE & NutE (D)
2. Batis	04/05/06/07/08	German	Breeder choice; High NupE & NutE (W)
3. Beaver	04/no/06/07/08	3	WGIN DH parent; High Canopy N requirement; Best NutE (W)
4. Cadenza	04/05/06/07/08	2	WGIN DH parent; Best NupE (W)
5. Claire NEW 2005	No/05/06/07/08	3	Biggest area on RL; WGIN DH parent; Good second wheat
6. Cordiale NEW 2006	No/no/06/07/08	2	Good second wheat
7. Hereward	04/05/06/07/08	1	Best protein on RL; benchmark bread variety
8. Hurley NEW 2005	No/05/06/07/08	1	Low NupE & NutE (W)
9. Istabraq NEW 2005	No/05/06/07/08	4	Best yield on RL; Distilling cultivar; In LINK 'GREENgrain'; Good second wheat
10. Lynx	04/05/06/07/08		Low NupE, high NutE (W)
11. Malacca	04/05/06/07/08	1	Biggest Group 1 area; DH choice; Low NupE, high NutE (W)
12. Maris Widgeon	04/05/06/07/08	1	Tall (rht), old cultivar
13. Mercia	04/no/06/07/08	1	Low NupE & NutE (desk); Low Canopy N requirement; In IGF micro-array
14. Monopol	04/05/06/07/08	German	Breeder choice; High NupE, worst NutE (W)
15. Napier NEW 2006	No/no/06/07/08	4	Good second wheat
16. Paragon	04/05/06/07/08	1	Spring variety; WGIN mutagenesis population; High NupE (W)
17. Riband	04/05/06/07/08	3	WGIN DH parent; Distilling cultivar; In LINK 'GREENgrain'; High NutE (W)
18. Robigus NEW 2005	No/05/06/07/08	3	Best Group 3 yield; Best NUE, high NupE & NutE (D); Good second wheat
19. Savannah NEW 2005	No/05/06/07/08	4	Best NutE (D)
20. Shamrock NEW 2005	No/05/06/07/08	1	High root length density at depth
21. Soissons	04/05/06/07/08	2	WGIN DH parent; Early maturing; High NupE, low NutE (W)
22. Sokrates	04/05/06/07/08	German	Breeder choice; High NutE (W)
23. Solstice	04/05/06/07/08	2	Biggest Group 2 area; DH choice; Worst NupE (W)
24. Xi19	04/05/06/07/08	1	Best Group 1 yield; High NUE, NupE, NutE (D); Low NupE (W)
Other genotypes used only in 2004 trial			Arche, Caphorn, Cappelle-Desprez, Chablis (Spring type), Einstein, Enorm, Flanders, Isengrain, Opus, Petrus, Rialto, Scorpion, Spark and Zyta

(W) WGIN-04 results with no fertiliser-N; 'low' ranked in lower quartile, 'high' ranked in upper quartile

(D) Defra NUE desk study 2005

NUE Nitrogen Use Efficiency (NupE x NutE)

NupE Nitrogen Uptake Efficiency (uptake/available)

NutE Nitrogen Utilisation Efficiency (yield/uptake)

In harvest year 1 (2004) the nitrogen rates were N0 (nil), N1 (50 kg/ha), N2 (200 kg/ha) and N3 (350 kg/ha)

In harvest year 2 (2005) the nitrogen rates were N0 (nil), N1 (100 kg/ha), N2 (200 kg/ha)

In harvest year 3 (2006) the nitrogen rates were N0 (nil), N1 (100 kg/ha), N2 (200 kg/ha)

Table 1: Wheat cultivars used in the WGIN Nitrogen Use Efficiency trials with a picture of the field trial

landraces from the Vavilov Institute (VIR) were selected from over one thousand available *T. monococcum* landraces based on their resistance/susceptibility to important Russian wheat pathogens and pests such as powdery mildew, leaf rust and aphids. In our collection most accessions originated from European, but a few were collected from West Asia, Eurasia and Africa. Considering the cultivation and domestication history of *T. monococcum*, over a quarter of the collections were of Turkish origin and were initially collected from various climate and cultivation zones in Turkey.

The collection has several noticeable features. For instance, the earliest collection dated back to the beginning of the last century (1904). Our collection includes a unique *T. monococcum* accession *PI355520* which was used as bridge species for successful trait introgression (*Cox et al., 1991, Plant Breeding 107: 105-118*). The 'elite' *T. monococcum* accession L118 which has unique semi-dwarf and other feature was kindly supplied by the Max Planck Institute (Benjamin Killian, Francesco Salamini and Maarten Koornneef). MDR050 has a unique ear phenotype of short and compact ear and no awns and had been selected from the progeny of a cross between *T. monococcum* and *T. sinskajae* (*Korzun, et al., 1998, Schr. Genet. Ressourcen Bd. 8, 244-247*). We also acquired the widely used accession *DV92* (renamed MDR308 in our collection) which had been used for the generation of a genetic map, construction of a BAC library (*Lijavetzky, et al., 1999, Genome 42, 1176-1182*) and cloning of several important genes (e.g., *Yan et al., 2003 PNAS 100, 6263-6268*). Within the WGIN project, the 24 landraces from VIR were genetically purified by single plant selection.

2. Generation of *T. monococcum* mapping populations

In the process of identifying and characterising novel traits in *T. monococcum* (see WGIN newsletter November 06 issue), it was observed that several accessions exhibited contrasting phenotypes in various traits including plant morphological and developmental traits, grain texture, resistance to major wheat UK pathogens and germination under salt and drought stresses.

Based on this information, we selected several representative *T. monococcum* accessions and generated five F_2 mapping populations (Table 3).

Female	Male	F_1 seeds	F_1 plants	F_2 seeds	F_3 seeds
MDR002	MDR043	22	6	2400	-
MDR043	MDR040	38	6	2400	100 for field phenotyping in 2007 at Rothamsted farm
MDR308 (DV92)	MDR002	18	3	1200	104 lines for Septoria leaf blotch resistance phenotyping
MDR308 (DV92)	MDR044	1	1	400	100 for field phenotyping in 2007 at Rothamsted Farm
MDR308 (DV92)	MDR043	43	3	1200	-

Table 3: Mapping populations generated for T. monococcum

These resources will facilitate the genetic dissection of many traits of interest. In addition, *T. monococcum* has often been used as a model species for wheat functional genomics employing a subgenome approach. For instance, a F_3 mapping population of MDR002 x MDR308 has been used to study the genetics of resistance to *Septoria tritici* leaf blotch (telemorph *Mycosphaerella graminicola*) in *T. monococcum* to identify a genetic locus controlling sporulation. Besides the five populations listed in Table 3, an additional 10 mapping populations will be constructed in 2007.

3. Wheat introgression lines

Ultimately, some of the useful traits identified in *T. monococcum* need to be transferred to bread wheat to facilitate genetic improvement. The prevalent existence of resistance to *Mycosphaerella graminicola* in *T. monococcum* may provide a new route to control this important leaf blotch disease in wheat. Two introgression routes have so far been tested. *T. monococcum* accessions MDR002 and MDR308 were used as male parents and were individually crossed to bread wheat varieties Chinese Spring, Riband and Cadenza which were the female parents. Several F_1 hybrids have been obtained through the use of either embryo rescue or direct seed-setting (Figure 2). The initial assessment of resistance to *M. graminicola* indicates that the resistance is retained in F_1 plants. We are in the process of backcrossing the F_1 plants to bread wheat to stabilise the resistant traits. The second introgression route takes advantage of the unique

Figure 2. Introgression of T. monococcum traits into T. aestivum. The F_1 hybrids exhibited hairy leaf (top panel), long awn (middle panel) and resistance to M. graminicola (bottom panel) phenotypes from the T. monococcum parent.

T. monococcum accession *PI355520* which contained two dominant genes and helps maintain fertility of F_1 introgression plants. The F_1 seeds and seedlings of several crosses employing *PI355520* have been obtained.

4. Chemically mutagenised populations

To use *T. monococcum* as a model for establishing trait : gene relationships, several chemically mutagenised populations have been generated using EMS (Table 4). The MDR050 EMS-mutagenised M_2 population was initially donated by Kay Denyer (JIC) and multiplied at Rothamsted. MDR050 has a large grain size and has been used for studying starch biosynthesis in cereal endosperm (personal communication, Kay Denyer). DV92 (MDR308) was selected as the second genotype for mutagenesis since many molecular genetic resources are available. M_3 ear rows will be observed in the field in 2007 for altered traits including resistance to various pathogens, plant growth and development and grain associated features. These populations will also form the basis to screen for novel variants of genes of interest using the TILLING technique (see WGIN Objective 9).

5. Ion-beam mutagenised populations

To improve the chances of identifying associations between genes and traits of interests, ion-bean radiation has been used to expand the spectrum of mutations (*Wu and Yu, 2001, Radiation and Environmental Biophysics **40,** 53-57*). Three separate populations of *T. monococcum* accession DV92 (MDR308) as well as the bread wheat cultivar Cadenza were treated with different dosages of N ions. Germination assays indicated that *T. monococcum* accession DV92 was less tolerant than Cadenza to the ion-bean mutagenesis procedure (Table 5).

Accession	M_1	M_2	M_3	Note
MDR050	3000	1500	1500	M_3 seeds for field phenotyping in 2007 at Hungary Academy of Science (Dr. Bedo Zoltan).
MDR308 (DV92)	3000	1800	1000	M_3 seeds for field phenotyping in 2007 at Hungary Academy of Science (Dr. Bedo Zoltan).

Table 4: EMS mutagenised T. monococcum populations

Wheat	Dosage	Total sown	Ungerminated	Germinated	Germination (%)
T. monococcum (MDR308)	$2x10^{16}$ N^+/cm^2	961	417	544	56.6
	$5x10^{16}$ N^+/cm^2	1206	510	696	57.7
	$8x10^{16}$ N^+/cm^2	1259	761	498	39.6
	Total	3246		1733	
T. aestivum (Cadenza)	$2x10^{16}$ N^+/cm^2	1268	34	1234	97.3
	$5x10^{16}$ N^+/cm^2	1164	45	1119	96.1
	$8x10^{16}$ N^+/cm^2	1172	100	1072	91.5
	Total	3604		3425	

Table 5: Ion-beam irradiated wheat populations

6. Construction of a SSR map for *T. monococcum*

Another activity pertaining to identifying phenotype : genotype relationships was the construction of a high-resolution SSR marker map for *T. monococcum*. A F_3 population consisting of 94 individuals from the cross MDR308 (DV92) x MDR002 (Table 4) was used to construct the first SSR marker linkage map for this species. Li-cor slab-gel based DNA analyser and fluorescence dye-labelled primers were used for high throughput multiplexing SSR marker analysis (Figure 3).

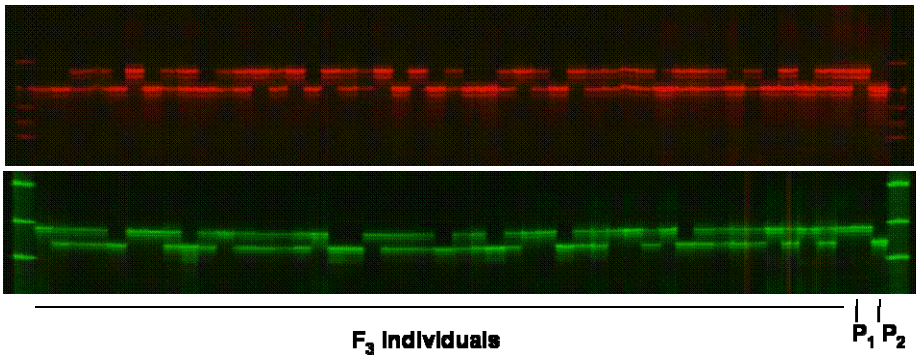

Figure 3. Genotyping of F_3 individuals and parental lines from the MDR308 x MDR002 cross. Shown are the PCR products amplified using IRD-labelled primers which were visualised with Li-cor DNA 4300 Analyser for the SSR markers wmc177 (red) and wms118 (green).

In total, 256 SSR markers mapped to the A genome of bread wheat were tested. So far we have assigned over 60 SSR markers onto the 7 chromosomes. In 2007, additional markers will be added to the map. Once constructed, the linkage map will facilitate association genetics for the identification of novel genes and variant alleles.

7. Establishing a TILLING platform

In conjunction with WGIN Objective 9, TILLING has been established as a reverse genetic platform for gene function studies in *T. monococcum* (see Objective 9 for details).

EMS and Gamma Mutated Paragon (JIC) Objective 7

Two populations of mutated Paragon have been developed at the John Innes Centre with Ethyl Methane Sulphonate (EMS) and Gamma irradiation treatments.

The largest and furthest developed population is that of EMS treatments. A population of 6500 lines has been developed to the M6 generation. Seed from all generations are held and are available, but the largest stocks are that of the M6 (also specimen ears) after the population was grown in field trial conditions. DNA of this population is also available, after extractions were made from the entire collection at the M3 generation. A smaller population of 2000 lines of Gamma irradiated material is available having been developed to the M3 generation.

Paragon seed was treated with EMS at varying concentrations for 16 hours until 50% germination was achieved. A 1% concentration was found to ensure numerous mutations whilst still maintaining a sizeable population. From 7000 treated seeds (M1), 3461 seeds were sown. These were grown to maturity in 1 l pots to give the first generation every chance possible to survive. One bagged ear was harvested and two seeds from this were sown bring the population to 6922 lines.

A Single Seed Descent (SSD) technique was used to rapidly develop the populations from M2 to M5. 96 well, small celled plant trays were used and filled with a peat and sand compost which ensured fast growing, single tillered plants (Figure 4). Under ideal conditions heading

Figure 4: Single seed descent of Paragon mutants

occurred after 8 weeks from sowing. With just one tiller, bagging was made problem free as all ears were bagged without duplication. The ears produced were mostly fertile and healthy although some sterility did occur at each generation. If one sibling was lost through this, a repeat sowing from the other was made to keep continuity in the population. Throughout growth and at harvest plant and ear phenotypes were noted and photographs archived.

Seed at the M5 generation was produced for the following season's field trial when 25 – 30 plants were grown per line under field trial conditions (without fungicides or PGR) instead of the previous one plant under glass. Sowing for this was carried out on March 16th 2006. Throughout the season, lines were scrutinised for mutations by regular field visits. Variant phenotypes were identified which had been successfully fixed into the Paragon background as plants within rows were mostly uniform for mutations expressed. For the complete field records visit www.wgin.org and follow directions to EMS Mutated Paragon Sort Sheet. Here select phenotypes of interest and use 'data sort' to find corresponding lines.

To give a general view on the diversity created in this mutagenesis experiment, heading or ear emergence occurred over almost a one month window. The vast majority of lines were late heading compared to Paragon (up to 25 days). However a few lines were early by up to four days. Height was also skewed, with far more short than tall lines. 25 lines (0.4%) were shorter than half the height of Paragon's 84 cm with 548 (8.4%) at least 10% shorter. Compare that to 133 (2%) of lines 10% taller than Paragon and only five lines were seen to be taller than the tallest Paragon heights recorded.

Figure 5 (left): Paragon mutant field trial showing early senescence line in foreground
Figure 6 (right): In contrast, a stay green mutant during ripening

Figure 7: Mutant height variation with Paragon third from the right

Other mutations noted included some mildew resistance, susceptibility to cold through zebra leaf expression, stay green and early senescence, discolouration to stems and leaves, low tiller number, ear morphology variation, flag leaf variants, peduncle lengths, awn suppressor knockouts, wax-less. Many more are available by reference to the WGIN website.

Figure 8: Four day earlier heading mutant

Figure 9: Rounded seed mutation on left with Paragon on right

Mutated lines with alterations in heading time, height, seed shape / size, ear morphology are currently being crossed with Avalon and Cadenza. This will give some information on the genetic control of these mutations.

Paragon seed was also mutagenised with Gamma irradiation. After an initial test at the Norfolk and Norwich University Hospital, doses of 150, 200 and 250 Grays were calculated to achieve the larger DNA knock outs whilst maintaining the appropriate germination rates. For the larger numbers of seed required for the project, seed was then sent to the International Atomic Energy Association in Austria (IAEA). It has since been noted that the 250 Grays dose was the most successful and lines treated with this dose have been the most useful as part of a PhD project investigating Rht allelic mutation rates.

TILLING (RRes) Objective 9

TILLING, a method for mutation detection and SNP discovery, has been successfully established in hexaploid wheat. This allows the rapid screening of lines from germplasm collections and mutated populations for novel alleles in specific candidate genes. In parallel, we have produced a population of wheat cv. Cadenza treated with the mutagen ethylmethanesulphonate (EMS) to generate variation throughout the genome. DNA samples were taken from the M2 population of ~4,500 individuals and M3 seeds has been archived. In addition, in collaboration with the EU HEALTHGRAIN programe, 2,200 M3 lines have been evaluated in the field, being scored for a wide variety of characters and revealing variation in plant height, time of ear emergence, ear glaucosity, ear shape, fertility and resistance to several diseases. Several groups are screening these lines for other characters.

The TILLING method relies on the detection of mismatches in heteroduplex DNA (Figure 10); the method is technically challenging but high-throughput.

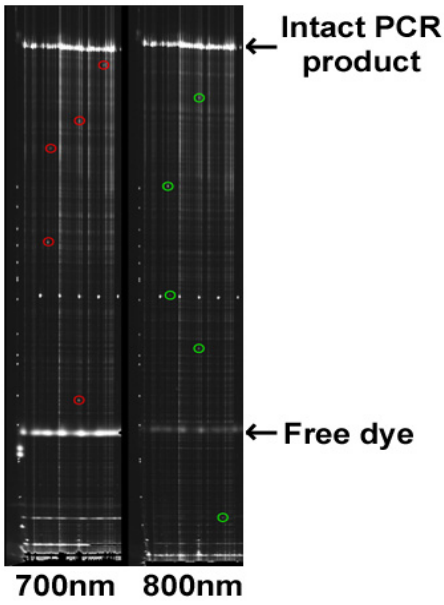

Figure 10: TILLING in 96 individuals (2-fold pooled) of the wheat (cv. Cadenza) EMS population for mutations in the TaGA20ox1A gene. Red and green circles indicate cleavage products in the sense and antisense DNA strands, respectively.

Using primers specific for individual homoeologues of the gibberellin 20-oxidase-1 gene, a candidate gene for resistance to pre-harvest sprouting, TILLING was used to identify point mutations in this gene (Figure 11), using a

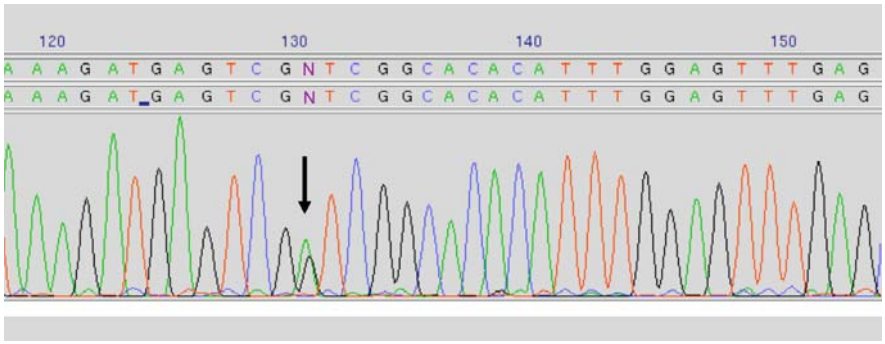

Figure 11: Novel mutation in a gibberellin 20-oxidase gene (TaGA20ox1A) detected by TILLING. This target is a candidate gene for resistance to pre-harvest sprouting.

robot to aid handling of the large number of samples. (Figure 12). A large number of novel

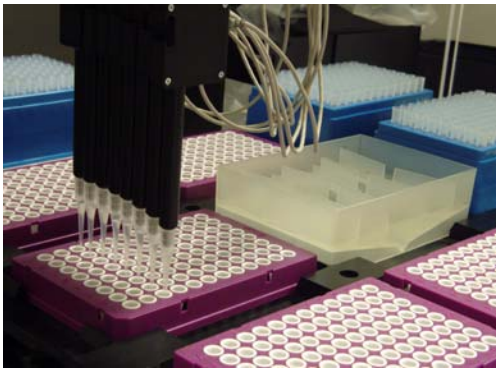

Figure 12: Beckman robot arraying DNA samples for TILLING

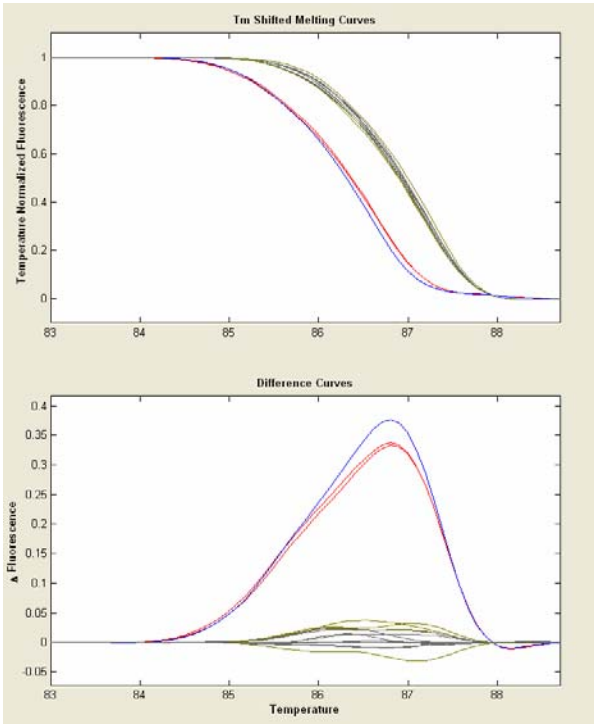

Figure 13: Mismatch detection in genes of interest using high-resolution DNA melting curve analysis. A 500bp PCR fragment of barley Rym4 gene was amplified from wild type (red and blue) and a mutant line carrying a C to T mutation created by EMS treatment. PCR was carried out in the presence of LC-green fluorescent dye and the products were subjected to melting curve analysis using the Lightscanner (Idaho Technology). The melting profiles (top) and melt difference curves (bottom) of the wild type and mutant are clearly different from each other, demonstrating that this technology could be used for mutation discovery and SNP detection.

alleles from the A and D homoeologues of this gene have been identified and the genotypes are being grown for phenotyping. TILLING has revealed a high mutation rate in the Cadenza EMS population, with each plant containing approximately one point mutation every 20 kbp. Thus, for a 1kbp target we would expect to find >200 novel mutants in our population, with a high probabililty of finding a range of knockout and knockdown mutations in all three homoeologues of any gene. This service is available to the research community for gene validation and allele mining.

We are also developing other methods for mutation discovery: the Lightscanner can detect point mutations in PCR products through high-resolution melt analysis (Figure 13). We have demonstrated that this can be used to distinguish wild-type and mutant wheat genes (Figure 14) and we aim to show that this can be more consistent, higher throughput and less costly than the established cleavage-based TILLING method.

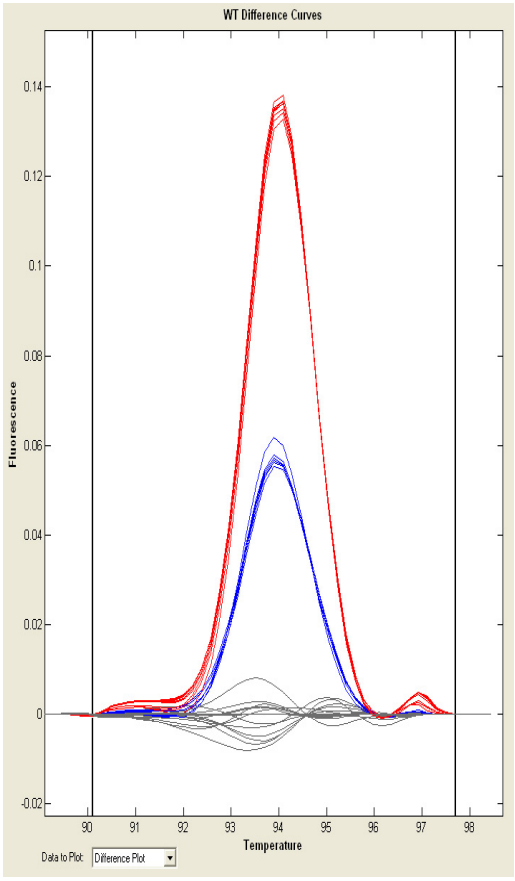

Figure 14: Mutation detection on the Lightscanner. Discrimination between DNA containing wild-type (black) or point mutant variants (blue and red) of the wheat GA20ox1A gene using homoeologue-specific primers and high-resolution melt analysis (fluorescence difference curves plotted against temperature).

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

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Next stakeholder workshop: November 2007 at Rothamsted Research