I·T·M·I

Tenth International Public Workshop

14–16 June 2000

University of Delaware
Clayton Hall
Newark, Delaware USA

Sponsored by

University of California
Genetic Resources Conservation Program for the
International Triticeae Mapping Initiative

and the

DuPont Company

PROGRAM, TEXTS, AND ABSTRACTS

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Services of the University of Delaware Office of Conference Services in support of this Workshop are gratefully acknowledged.

The **International Triticeae Mapping Initiative**, formed in 1989, is an informal consortium of geneticists dedicated to developing recombination maps of the genomes of the species in the Triticeae tribe of the Poaceae family. The informal nature of ITMI was meant to encourage independent research among the primary participating laboratories and at the same time encourage collaboration so that good maps would be obtained rapidly and materials and resources could be shared. The progress in building such recombination maps has been steady and the objectives of ITMI have been achieved for the major crop species in Triticeae. The momentum and data from these maps have been a major impetus for the increasing interest in genomics and comparative mapping both within Triticeae and between species of Triticeae and those of other grass tribes.

PROGRAM

Tuesday, June 13, 2000

Arrival and registration

3:00p **Registration available until 10:00p, Clayton Hall**

7:00 **NSF-Wheat Genomics Project Investigator Meeting, Rm. C123, Clayton Hall**

Wednesday, June 14, 2000

7:00a **Registration available, Clayton Hall**

7:30 **Access to hall for poster setup**

8:00 **Opening/Welcome/Announcements**

Session 1—EST/Genomics projects

Chair: ***Mike GALE, John Innes Centre, Norwich UK***

8:15 French national/industry consortium program in wheat genomics

Philippe JOUDRIER[1] and **Eric HUTTNER**[2]

[1]*INRA, Unité de Biochimie et Biologie Moléculaire des Céréales, 2, Place Viala 34060 Montpellier, Cédex 01, FRANCE*

[2]*RhoBio–A Joint Venture between Rhône-Poulenc Agro and Biogemma, 2, rue Gaston Crémieux, CP5707, 91057 Evry, Cédex 07, FRANCE*

8:45 Canadian wheat genome program

Daryl SOMERS

Agriculture and Agri-Food Canada, Cereal Research Centre, 195 Dafoe Rd., Winnipeg, MB R3T 2M9 CANADA

9:15 UK wheat and barley genome programs

Robbie WAUGH

Genetics Division, Genomics Unit, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA SCOTLAND

9:45 Break and poster viewing (30 min.)

10:15 Australian wheat and barley genome programs

Rudi APPELS

Div. of Plant Industry, CSIRO, GPO Box 1600, Canberra City, ACT 2601 AUSTRALIA

10:45 Comparative sequence analysis of cereal genomes

Jeff BENNETZEN

Dept. of Biological Sciences, Purdue University, West Lafayette IN 47907 USA

11:15 US wheat genomics

Olin ANDERSON

USDA ARS WRRC, 800 Buchanan Street, Albany, CA 94710, USA

11:45 Lunch break and poster viewing (90 min.)

1:15p Summary of May 2000 SCRI plant genomics meeting

Peter LANGRIDGE

Dept. of Plant Science, University of Adelaide, Waite Campus, Glen Osmond, SA 5064 AUSTRALIA

1:45 General discussion: Progress, linkages, collaboration

2:30 Break and poster viewing (30 min.)

Session 2—Molecular markers

*Chair: **Mark SORRELLS**, Cornell University, USA*

3:00 Development and application of wheat microsatellite markers (Abstract, p. 10)

Marion S. RÖDER, Viktor KORZUN, Elena PESTSOVA, Andreas BÖRNER & Martin W. GANAL
Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, 06466 Gatersleben, GERMANY

3:20 Variety identification and characterization of germplasm using wheat microsatellite markers

Martin GANAL
Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, 06466 Gatersleben, GERMANY

3:50 Development and use of some PCR-based molecular markers for gene tagging, mapping, and diversity studies in bread wheat (Abstract, p. 7)

P.K. GUPTA
Molecular Biology Laboratory, Department of Agricultural Botany, Ch. Charan Singh University Meerut-250 004, INDIA

4:10 Wheat gene responding to feeding by avirulent first-instar Hessian fly larvae (Text, p. 10)

Christie E. WILLIAMS[1,2], Chengzhi LIANG[2], Sue E. CAMBRON[2], Jill A. NEMACHECK[2] and Chad C. COLLIER[1,2]
[1]USDA-ARS Crop Production and Pest Control Research Unit, West Lafayette IN 47907 USA
[2]Department of Entomology, Purdue University, West Lafayette, IN 47907 USA

4:30 Poster viewing

5:15 **Wine and Cheese Reception**

7:00 **ITMI Coordinator Meeting, Rm. 123 Clayton Hall**

Thursday, June 15, 2000

Session 3—Structural and functional genomics (Speakers from DuPont Company)

*Chair: **Wayne POWELL***

8:00a Strategic directions for genome science research
S. TINGEY

8:20 Large scale EST gene discovery programs
M. DOLAN

8:50 SSR discovery & implementation programs
P. WOLTERS

9:20 Gene expression analysis
A. RAFALSKI

9:50 Physical mapping in maize
M. MORGANTE

10:20 **Break and poster viewing (40 min.)**

11:00 **Transportation to DuPont Company genomics labs**

11:30 **Tour sequencing and genotyping facilities**

12:30p **Transportation to Longwood Gardens (Packed lunch)**

5:00 **Return to University of Delaware campus**

6:30 **Workshop dinner**

Friday, June 16, 2000

Session 4—Functional genomics: Methods and applications

Chair: ***Jan Dvořák****, University of California-Davis, USA*

8:30a Plant genome analysis using methyl filtration

W. Richard McCombie
Cold Spring Harbor Laboratory, 1 Bungtown Rd., Cold Spring Harbor, NY USA

9:15 Functional genomics of growth and end-use quality in cereals

Geoff Fincher
Dept. of Plant Science, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, AUSTRALIA

10:00 Break and poster viewing (30 min.)

10:30 SSR and SNP markers derived from barley ESTs (Text, p. 8)

R.J. Henry, T.A. Holton, R. Kota, A. Muirhead, L. McClure, and G. Ablett
Cooperative Research Centre for Molecular Plant Breeding, Centre for Plant Conservation Genetics, Southern Cross University, PO Box 157, Lismore NSW 2480, AUSTRALIA

10:50 Investigating starch biosynthesis in wheat (Abstract, p. 5)

Diane M. Beckles and Karen E. Broglie
DuPont Agricultural Biotechnology, Experimental Station, Wilmington, DE 19880-0402 USA

11:10 QTLs and major genes for wheat seed dormancy (Abstract, p. 6)

John Flintham
John Innes Centre, Norwich Research Park, Norfolk NR4 7UH UK

11:30 The molecular dissection of the *Ph1* locus (Abstract, p. 6)

Simon Griffiths, Caroline Dalgliesh, Tracie N. Foote, Katherine S. Henderson, Wayne Powell, and Graham Moore
Cereals Research Dept, John Innes Centre, Colney, Norwich, NR4 7UH UK

11:50 Lunch break and poster viewing (100 min.)

Session 5—Disease resistance

Chair: ***Bikram Gill****, Kansas State University, USA*

1:30p Differentially expressed genes in wheat spikes after scab inoculation (Abstract, p. 13)

Yang Yen[1], D.-H. Xing[1], Y. Jin[2], and J. Rudd[2]
[1]Dept. of Biology and Microbiology and [2]Dept. of Plant Science South Dakota State University, Brookings, SD 57007 USA

1:50 Genetic and physical mapping at the *Pm3b* locus of powdery mildew resistance in wheat (Abstract, p. 12)

Nabila Yahiaoui, Catherine Feuillet, Zhong-Da Liu, and Beat Keller
Institut für Pflanzenbiologie, Universität Zürich, Zollikerstr., 107, 8008 Zürich SWITZERLAND

2:10 Partial sequencing of a cDNA library of Fusarium-infected wheat spikes

John Fellers
USDA-ARS-PSERU, Dept. of Plant Pathology, Kansas State University, 4006 Throckmorton Hall, Manhattan, KS 66506 USA

2:30 Break and poster viewing (30 min.)

3:00 **Session 6—General ITMI Business Meeting**

Chair: ***C.O. Qualset****, GRCP, University of California, USA*

5:30 **Adjournment, poster take down**

TEXTS OR ABSTRACTS OF ORAL PRESENTATIONS

Listed alphabetically by presenting author (**in boldface**)

Investigating starch biosynthesis in wheat

Diane M. BECKLES and Karen E. BROGLIE

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The starch accumulated in plant heterotrophic organs plays a significant role in the human diet since it can account for as much as 80% of man's calorific intake[1]. In addition, both native and modified starches are increasingly being used in various manufacturing industries—as a thickener, texturizer, and stabilizer in the food industry, in the textile and paper industry, and in the manufacture of ethanol, biodegradable plastics, paints, and glues[2]. Wheat starch in particular is unique—it is the only type of starch that produces a suitable leavened product[3]. In spite of the importance of starch (especially wheat starch) to man, our present knowledge of how the starch granule is initiated and assembled, and what determines the final size and shape of the granule is poor. We therefore decided to take a whole genome and proteome approach in order to investigate these, and any hitherto unknown factors that may play a role in wheat starch biosynthesis.

Wheat endosperm starch potentially provides a good model for studying the process of granule initiation, and for elucidating the genetic determinants of starch granule size and shape. Wheat starch occurs in two morphologically distinct forms. One population is lenticular in shape, ranges in diameter from 10 to 50 µm, and is deposited at the earliest stage of wheat grain development. This population is commonly referred to as A-granules. The other population consists of spherical, smaller (1 to 10 µm) granules, which are initiated about 10 to 14 days after anthesis. These are called B-granules and can account for 25 to 40% of the total volume but more than 90% of the total number of starch granules. Since these two types of granules have different physico-chemical properties, an understanding of the factors that influence their relative proportions may allow this characteristic to be manipulated to produce wheat starches with novel properties. By comparing the gene expression profile in wheat endosperm before and after synthesis of B-starch granules, we hope to identify factors important in the initiation of this class of granules. Moreover, since the period of B-starch granule initiation also coincides with the phase of rapid starch accumulation in the endosperm, it should be possible to delineate factors important to starch biosynthesis as a whole.

The expression profile of genes in wheat endosperm before and after the initiation of B-granules was interrogated using cDNA microarrays and Lynx technology. In the cDNA microarray experiment, the expression of a finite number (about 1000) of selected genes was monitored. In contrast, Lynx technology allows one to look at potentially **all** of the genes that are differentially expressed between these two types of tissue. We are also taking a proteomic approach towards studying B-granule formation. The amyloplast is the organelle in which starch is made. We have prepared amyloplast-enriched fractions from wheat endosperm at the stage of development when only A-granules are present, and when B-granules are initiated, in an effort to concentrate proteins potentially involved in starch biosynthesis in these two types of tissues. By comparing the catalogue of proteins isolated from these tissues, we hope to find novel, differentially expressed proteins which may be involved in B-starch granule synthesis. The progress we have made on this work will be discussed.

[1]GALLIARD, T. 1987. Starch availability and utilisation. pp 1–14. *In:* T. Galliard (ed.) *Starch: Properties and Potential.* Society of Chemical Industry. John Wiley & Sons, Chichester, UK.

[2]SHREWMAKER, C.K. and D.M. STALKER. 1992. Modifying starch biosynthesis in potatoes. *Plant Physiology* **100**:1083–1086.

[3]HOSENEY. R.C., K.F. FINNEY, Y. POMERANZ, and M.D. SHOGREN. 1971. Functional (breadmaking) and biochemical properties of wheat flour components. VIII. Starch. *Cereal Chemistr*y **48**:191–201.

QTLs and major genes for wheat seed dormancy.

John FLINTHAM

John Innes Centre, Norwich Research Park, Norfolk NR4 7UH UK

Triplicate gene control of wheat grain colour was the first described example of polygenic inheritance affecting a quantitative character—seed dormancy. Although there appears to be a correlation between the dosage of dominant (red) alleles and dormancy, this effect is small compared to the difference between red and white wheats. Furthermore, considerable genetic variance remains unexplained by the *R* gene model. In red and white NILs, some of this residual variation is associated with the basal dormancy in the absence of red pigment; additional differences are associated with the response to introgression of dominant *R* alleles.

Aneuploid analyses have indicated that wheat Group 3 chromosomes carry other dormancy genes in addition to the *R* loci. Candidate genes for dormancy (*TaVp1*) and for flavonoid pigment synthesis (*TaDfr*) also map to these chromosomes. This raises the possibility that the apparent dosage effects, associated with dominant *R* alleles in earlier studies, may have resulted from genetic linkage.

Mapping studies have revealed multiple QTLs unrelated to grain colour, usually with different loci detected in different populations and / or environments. A summary of QTLs mapped recently at JIC is given, together with the location of a novel, major dormancy gene on chromosome 4A. This new dormancy gene exerts its effect via the embryo (not the seed coat) and specifically affects the rate of loss of dormancy during after-ripening.

The molecular dissection of the *Ph1* locus

Simon GRIFFITHS, Caroline DALGLIESH, Tracie N. FOOTE, Katherine S. HENDERSON, Wayne POWELL, and Graham MOORE

Cereals Research Dept, John Innes Centre, Colney, Norwich, NR4 7UH UK

The *Ph1* locus of hexaploid wheat (*Triticum aestivum* L.) imposes a high level of stringency on chromosome pairing so that only homologous chromosomes pair during meiosis. It is this phenotype that has attracted a great deal of attention from wheat researchers over the past forty years. However, the underlying mechanism of this effect is not known. Other floral development phenotypes associated with the *ph1b* deletion include; reduced seed set, reduced seed weight, low levels of pollen production, and early flowering. We are interested in all of these effects because of their possible role in the control of chromosome pairing and because they are all traits of agronomic interest. Therefore, our aim is to fully characterise the *Ph1* locus by a process of molecular dissection.

Previously we have described the construction of a 4Mb YAC contig from the *Ph1* equivalent region of rice chromosome 9, and the isolation of six novel *ph1* deletion mutants, selected by the absence of one or more of three chromosome 5B-specific markers distributed across the locus. Using these mutants the *Ph1* equivalent region of rice was reduced to an estimated 400kb. Here we describe the next stage of our work.

In collaboration with DuPont a BAC contig from the equivalent region of rice has been assembled and a minimum tiling path of three BACs shotgun sequenced to 6x coverage. As a result of this effort approximately 30 putative rice genes have been identified. Generally, PCR products from these genes cross-hybridise well to wheat showing that they are highly conserved at the sequence level. As a result, Southern blots of the new *Ph1* deletion lines are easy to score for the presence or absence of the homologous wheat genes. These data allowed the reduction of the *Ph1* equivalent region of rice to ~150kb, and showed that gene order was well conserved between the two species at this locus.

The 150kb region contains ten predicted genes with homology to transcription factors, receptor kinases, metabolic enzymes, anonymous ESTs, and hypothetical genes from other species. We are now in the process of cloning the wheat homologues of these genes. This will provide us with the means to screen for more deletions in the region, with a level of resolution sufficient to account for all the phenotypes associated with the deletion of the *Ph1* locus.

Development and use of some PCR-based molecular markers for gene tagging, mapping, and diversity studies in bread wheat

P.K. GUPTA

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During the last four years, a variety of molecular markers in bread wheat were tried by us at Meerut, with the following two major objectives in mind: (i) development of molecular markers for marker aided selection (MAS) for three quality traits (high protein content, pre-harvest sprouting tolerance, and seed weight); (ii) germplasm characterization and analysis of genetic diversity among 55 elite genotypes. The mapping populations (recombinant inbred lines or RILs) for three traits were prepared by our collaborators at PAU, Ludhiana and the germplasm for diversity studies was procured from DWR, Karnal. RFLPs, RAPDs, in-gel hybridization, MP-PCR, and DAF, did not prove useful in bread wheat, due to bigger genome size (18,000 Mb), presence of homoeoloci in three genomes, high level of repetitive DNA, and the consequent low level of reproducibility. Therefore, more efficient marker systems like STMS, STS, AFLP, and SAMPL were successfully utilized for tagging as well as for the study of genetic diversity. Among the five molecular markers developed for marker aided selection, there were two STMS markers for protein content, one STMS and one STS marker for pre-harvest sprouting tolerance, and one STMS marker for seed weight. Marker validation was also undertaken for protein content utilizing three sets of 10 near isogenic lines (NILs) that were developed from three different crosses made at PAU, Ludhiana. The markers, developed as above, were also assigned to their respective individual chromosomes and their arms, utilizing nullisomic-tetrasomic lines and the ditelocentric lines. At least three of these markers were also physically mapped on 2DL, 6BS, and 7DL, using overlapping deletion stocks. Integrated physical maps for these three arms were also prepared using additional markers that were either available for physical mapping or were earlier mapped elsewhere.

For germplasm characterization and genetic diversity studies, 20 different STMS primer pairs, when used with all the 55 genotypes, detected 155 alleles at 21 loci. At individual loci, 1 to 13 alleles were observed, giving an average of 7.4 alleles per locus. $(GT)_n$ microsatellites were found to be the most polymorphic; polymorphic information content (PIC) was 0.71 and the marker index (MI) was 0.70. Of the 55 genotypes, 48 could be characterized using only 12 of the 20 primers used in this study. AFLP (using 8 primer pair combinations) and SAMPL (4 primer pair combinations) were also successfully utilized for similar genetic diversity studies using the same 55 genotypes. In yet another novel approach, primers based on retroposons were used in combination with *Mse*I primers of AFLP, which gave equally interesting results. The results from different marker systems differed, thus questioning the relative utility of individual molecular

marker systems for genetic diversity studies. In view of this, we are now using more than one marker system, along with data on morphological traits for estimation of genetic diversity, which should hopefully give a reliable estimate of genetic diversity.

SSR and SNP markers derived from barley ESTs

R.J. HENRY, T.A. HOLTON, R. KOTA, A. MUIRHEAD, L. MCCLURE, and G. ABLETT

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DNA sequence information is providing a growing resource for the identification of genetic markers for use in plants. The establishment of ITEC has made significant numbers of ESTs available for wheat and barley. EST databases have provided an important source for SSR or microsatellite markers from a range of plant species. SSR markers derived from ESTs may have advantages over those from traditional enriched genomic libraries. Wider transferability of these SSRs may be possible because of higher sequence conservation within ESTs. Mapping of the SSR provides a map location, in many cases, for a gene of known function. Single nucleotide polymorphism markers can also be designed from EST information. This provides a rich source of DNA polymorphism for use in genome mapping. We have designed molecular beacons to detect SNPs in barley. The relative value and potential application of SSR and SNP markers in barley will be discussed.

Background

Microsatellite markers are valuable in genotyping and evaluation of genetic resources (WU and TANKSLEY 1993; SAGHAI MAROOF et al. 1994; GARLAND et al. 1999: MAGUIRE et al. 1999). ITMI initially focused on the mapping of genomes in the Triticeae using RFLP markers. More recently microsatellite or SSR markers have been more widely used because of their ease of analysis. These microsatellites have mostly been isolated from enriched genomic libraries. This process is laborious and expensive. The growing availability of DNA sequence information provides new opportunities for the development of molecular markers for use in the Triticeae. The recent completion of the sequence of the rice genome provides an important source of data. The International Triticeae EST Consortium (ITEC) provides a new source of species-specific sequence information from which markers can be derived. We have investigated the identification of microsatellite and single nucleotide polymorphism (SNP) markers in EST sequences.

Two main options for the development of microsatellite markers are to source them directly from the target species or to identify them based upon information from a closely related species. Research at the Centre for Plant Conservation Genetics at Southern Cross University has addressed the isolation and characterisation of microsatellite loci from many plant species. Large numbers of loci have been identified in some species by producing enriched genomic libraries (ROSSETTO et al. 1999). In some cases such as sugarcane this has required optimisation of the microsatellite enrichment technique (CORDEIRO et al. 1999). Other species, such as pine trees have required screening of enriched libraries to allow efficient recovery of microsatellite loci (SCOTT et al. 2000a). Microsatellites derived from genomic libraries have been difficult to transfer between species in many cases, although exceptions have been found in some plant groups. For example, microsatellites appear to be widely transferable within the Myrtaceae, possibly due to limited sequence divergence in this family (ROSSETTO et al. 2000). Microsatellite transfer between *Pinus* species has also proven to be a useful option. Transfer of microsatellites within the cereals has been more limited (reference). One advantage of microsatellites derived from ESTs is the potential that they may be more transferable between species. Recently we demonstrated the potential to transfer microsatellite derived from grape ESTs to related species (SCOTT et al. 2000b). We are currently investigating transfer of sugarcane microsatellites derived from ESTs to sorghum and other related species compared with those from the International Consortium for Sugarcane Biotechnology (ICSB).

Microsatellites

The ITEC database was searched for microsatellite sequences and the loci identified are being evaluated for wheat and barley. Preliminary results with wheat indicate low levels of polymorphism for the microsatellites in wheat sequences. This may be due to the types of cDNA libraries represented currently in the ITEC database. We have evaluated 29 SSRs from barley EST sequences. These barley SSRs have also been evaluated in wheat. Most barley SSRs performed well, in contrast to a similar set of wheat SSRs derived from ESTs.

Single nucleotide polymorphisms

Single nucleotide polymorphisms have been detected by analysis of the Genbank DNA database. Comparison of EST sequences from a cDNA library derived from the variety Alexis and the sequences in the database allowed the detection of possible SNP loci. In other cases, over 50 SNP primer pairs have been designed and used to amplify from 10 barley genotypes to reveal potential SNPs. The initial application of these results has been in the design of variety specific assays for use in identification of barley genotypes.

Molecular beacons

The molecular beacon technique was demonstrated to work effectively in the analysis of a transgene in barley (KOTA et al. 1999). This work is being extended to the application of molecular beacons to the detection of SNPs in barley. Variety specific molecular beacons are likely to have wide application in industry.

Acknowledgements

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ROSSETTO M., A. MCLAUCHLAN, F.C.L. HARRIS, R.J. HENRY, P.R. BAVERSTOCK, L.S. LEE, T.L. MAGUIRE, and K.J. EDWARDS. 1999. Abundance and polymorphism of microsatellite markers in the tea tree (*Melaleuca alternifolia*, Myrtaceae) *Theor Appl Genet* **98**:1091–1098.

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Development and application of wheat microsatellite markers

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We continue to develop new microsatellite markers for the wheat genome and to place them on the mapping population of the International Triticeae Mapping Initiative (ITMI). To date we have mapped 410 functional primer pairs resulting in 498 mapped loci. Sixty of these loci belong to primer pairs which were isolated from *Triticum tauschii*, the D-genome donor of cultivated wheat. As expected these primer pairs map predominantly to the D-genome of bread wheat. Primer pairs which are monomorphic on the ITMI population are assigned to chromosomes using nulli-tetrasomic stocks of Chinese Spring.

The growing number of wheat microsatellites allows a variety of practical applications including germplasm and varietal surveys, verification of substitution lines and other cytogenetic stocks, as well as mapping of genes.

We successfully used microsatellites to locate a novel resistance gene on chromosome arm 3BS in a segregating population. The resistance gene confers a non-race specific durable field resistance against yellow rust. The mapping of further non-race specific resistance genes against yellow rust and leaf rust is under way.

In another project, a closely linked microsatellite was found for the dwarfing gene *Rht8*. This marker is diagnostic for different phenotypes of *Rht8* and was used to study the distribution of the *Rht8* alleles in varieties and germplasm.

Wheat gene responding to feeding by avirulent first-instar Hessian fly larvae

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Abstract: The relationship between resistant wheat and avirulent first-instar Hessian fly larvae can best be described as a classic gene-for-gene response (HATCHETT and GALLUN 1970). Resistance is believed to involve an interaction between a specific gene product of the fly and the product of a corresponding gene in the host. This incompatible interaction results in a localized hypersensitive response (SHUKLE et al. 1992) at the site of attempted feeding near the base of the plant and leads to larval death within a few days. However, we have identified a defense-response gene that is induced in distant leaves within a few hours of attempted feeding, indicating a systemic component of this interaction. The amino acid sequence derived from the partial coding region of this gene, *Whi-1* (Wheat Hessian fly Induced gene), is similar to several others that are induced by BTH (benzothiadiazol) and jasmonic acid, both systemic inducers of defense-response genes. The c-terminal end of the sequence resembles jacalin-like lectins and other proteins that bind carbohydrates and may play a role in defense against insects and microbial pathogens.

Introduction: The relationship between wheat and the Hessian fly is one of the best-studied plant/insect interactions. Over 29 resistance genes have been identified in wheat and its relatives, and several have been used extensively in cultivars for the past 60 years as a successful form of host-plant resistance (RATCLIFFE and HATCHETT 1997). The rapid development of virulent Hessian fly populations, within 10 years of the deployment of new resistance genes (PATTERSON et al. 1990) is considered to be the direct result of selection pressure imposed on the insect by vast acreage of single resistant wheat cultivars (HATCHETT and GALLUN 1968).

Incompatible interactions between wheat and the Hessian fly are characterized by death of avirulent first-instar larvae within three to five days of hatching (PAINTER 1930). Little phenotypic change is apparent in resistant plants, other than an occasional localized hyper-sensitive response (SHUKLE et al. 1992). Although avirulent first-instar larvae repeatedly attempt to feed on their host, they fail to establish a permanent feeding site (GROVER 1995).

Genetic studies have shown that wheat resistance and the Hessian fly avirulence both segregate as single dominant loci (RATCLIFFE and HATCHETT 1997). Many of the wheat resistance genes have been mapped and only two appear to be so tightly linked as to suggest allelism (OHM and DWEIKAT, unpublished; WILLIAMS and COLLIER, unpublished). Complementation tests show that 8 independent populations of Hessian fly, all of them virulent to resistance gene *H6*, each defeat the plant by variation at a common locus (Williams and Stuart, unpublished). This one-to-one correspondence between each wheat resistance gene and the matching insect avirulence gene fits the classical description of the gene-for-gene interaction that is commonly applied to plants and their microbial pathogens.

Recognition specificity in gene-for-gene interactions is conferred by the plant resistance genes. The recognition event leads to signal transduction, gene expression and containment or death of the pathogen. Our research characterizes one of the genes that quickly responds to feeding by avirulent Hessian fly larvae. The gene sequence suggests that it may play a role in plant defense.

Materials and Methods: Plant Tissues and Infestations: Wheat seedlings containing the *H9* gene for Hessian fly resistance (cultivar 'Iris') were infested with newly emerged adult Hessian flies of biotype L (avirulent on wheat containing *H9*) or *vH9* flies (virulent on *H9* wheat (MAAS et al. 1989). RNA Isolation for Differential Display: One week after infestation, leaves were collected and total RNA was isolated with the kit CLONsep 2 (Clonetech Laboratories, Inc., Palo Alto, CA). Differential display experiments were conducted according to the directions in the RNAimage kit 2 (Genhunter Corporation, Nashville, TN). BLAST searches: Searches were conducted with the BLASTn option to compare DNA sequences and the BLASTp option for protein sequences derived from the experimentally determined *Whi-1* partial cDNA sequence.

Results and Discussion: Differential display (LIANG and PARDEE 1992), yielded a 583 bp partial cDNA fragment corresponding to a wheat gene (*Whi-1*: wheat Hessian fly-induced gene) whose mRNA levels increased rapidly within 22 hrs of attempted feeding by avirulent Hessian fly larvae. The level of *Whi-1* mRNA increased for approximately 48 hrs in these incompatible interactions, and then began to decrease. First-instar Hessian fly larvae begin to die on resistant plants within three days of the initiation of feeding, corresponding well with the kinetics of *Whi-1* expression. *Whi-1* showed low level constitutive expression in uninfested plants and those undergoing a compatible interaction.

Sequence analysis of the *Whi-1* partial cDNA clone revealed an 82% identity to the 3' half of the coding region for the wheat chemically-induced gene *Wci-1.* The chemical BTH (benzo-thiadiazole) is a systemic inducer of *Wci-1* and other genes whose expression leads to systemic resistance to powdery mildew, leaf rust and septoria leaf spot (GORLACH et al. 1996). BLAST searches of the derived partial amino acid sequence from *Whi-1* identified jasmonate-induced genes (53-28% identity) and the alpha-chain of jacalin-type lectins (37-36% identity). Jasmonate, a signal molecule in wounding and induced systemic resistance (FARMER and RYAN 1992), induces a set of genes with little structural similarity. Jacalin-type lectins are involved in plant defense and some exhibit insecticidal properties (MURDOCK et al. 1990).

The structure, timing and systemic nature of *Whi-1* expression are suggestive of defense-response genes. However, its involvement in resistance to the Hessian fly is questionable. During the defense response, the plant's detection of avirulent larvae does not result in systemic resistance to subsequent infestation by other Hessian flies, as would be expected if *Whi-1* were phytotoxic. After the initial infestation by avirulent larvae, subsequent feeding by virulent larvae leads to obviation of resistance, allowing survival of both virulent and avirulent larvae (GROVER et al. 1989; STUART, personal communication). This phenomenon is quite different from systemic

acquired resistance in which an initial infection rallies plant defenses against future attack by a variety of pathogens, including the original. Rescue of avirulent larvae by obviation, so long after the initial infestation, suggests that avirulent larvae die by starvation rather than due to toxin ingestion. Thus, resistance mechanisms may be highly localized at the attempted feeding sites of avirulent larvae and may not prevent nearby virulent larvae from initiating systemic changes in the plant that result in obviation and plant stunting.

Although the function of *Whi-1* in plant defense is unclear, its response to avirulent Hessian fly larvae is the result of a systemic signal that is initiated during a gene-for-gene interaction that acts locally. Because increased *Whi-1* mRNA levels were not detected in incompatible interactions, the response is specific and unlike the induction of genes responding to generalized wounding by chewing insects.

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Genetic and physical mapping at the *Pm3b* locus of powdery mildew resistance in wheat

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Powdery mildews, caused by obligate biotrophic fungi, are responsible for some of the most economically important diseases of agricultural crops and particularly of cereals. In wheat (*Triticum aestivum* L.), powdery mildew is caused by the fungus *Erysiphe* (syn. *Blumeria*) *graminis* DC. *f. sp. tritici* (em. Marchall) and race-specific resistance to this pathogen is controlled by *Pm* genes. The *Pm3* locus consists of at least 6 different alleles or very close loci (ZELLER *et al.* 1993, *Euphytica* **68**:223–229) and was first mapped through classical genetic analysis on chromosome 1AS (MCINTOSH and BAKER 1969, *Proc Linn Soc NSW* **93**:232–238) in the same region as the leaf rust resistance gene *Lr10*. Near-isogenic lines were used to identify closely linked RFLP markers. A wheat probe (Whs179) and a barley probe (BCD1434) were

determined to be at 3.3 ±1.9 cM and at 1.3 cM respectively from *Pm3b* (HARTL *et al.* 1993, *Theor Appl Genet* **86**:959–963; MA *et al.* 1994, *Genome* **37**:871–875).

As a first step of a map-based cloning strategy for the isolation of the *Pm3b* gene, a mapping analysis was done on a segregating population of 176 F_2 plants from a cross between the near isogenic lines Chul/8*Chancellor (carrying the resistance gene *Pm3b*) and Chancellor (susceptible genotype). Then, 2000 plants, a segregating F_2 population of the same cross, was grown. After infection and phenotype determination, fine mapping was started on the 477 susceptible F_2 plants. Wheat and barley RFLP probes as well as wheat microsatellites were tested for genetic linkage to *Pm3b*. The resulting genetic map showed a clustering of 7 tested markers at the *Pm3b* locus and no marker was found at the distal part of the *Pm3b* gene. The clustering of the probes being due either to very close physical distance between the tested probes and/or to a very low level of recombination in the region of interest, two strategies were followed to analyze and overcome this clustering. New segregating populations for the *Pm3* gene using the *Pm3b* donor line Chul and different susceptible lines were developed, and a physical analysis of the *Pm3b* region using large-insert libraries was started.

The first results on 480 F_2 plants from the cross Chul × Frisal showed a high level of recombination in the *Pm3b* region and most of the previously clustered markers could be mapped at different positions, proximal to *Pm3b*. The closest probe to *Pm3b* maps at 0.9 cM of the gene. Probes located at the distal end of chromosome 1DS of *Aegilops taushii* have been tested on this population and allowed to find a flanking marker at 4 cM distal to the gene.

The physical characterization of the *Pm3b* region was started by using a *Triticum monococcum* BAC library. The A genome of the diploid *T. monococcum* is close to the A genome of hexaploid wheat and should therefore be suitable for the establishment of BAC contigs in the region of interest on chromosome 1AS and to derive new probes for the high-resolution mapping.

Several strategies were used to derive mapable anchor probes from the BAC clones and the first results of the physical characterization of the *Pm3* region will be presented. These results showed that the obtention of anchor probes from the *T. monococcum* library is possible and that a strategy of chromosome walking using the *T. monoccocum* library could be feasible to isolate the *Pm3* gene from hexaploid wheat.

Differentially expressed genes in wheat spikes after scab inoculation

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Differentially expressed genes during scab development were investigated for spring wheat (*Triticum aestivum* L.) cultivars Sumai 3 (scab-resistant) and Wheaton (scab-susceptible). High quality total RNA was isolated from spikelet samples collected at 0 1, 16, 32, and 64 hours after inoculation with a drop of distilled water or *Fusarium graminearum* (isolate Fg4) conidiospores (70,000 spores/ml). A total of 14,502 differentially expressed sequence tags (ESTs) were revealed by mRNA differential display technique, with an average of 40.28 (17-60) per primer set. Of these ESTs, 382 are differentially expressed among the treatments, an average of 3.2 (0-14) per primer set. Our preliminary data revealed several types of gene expression patterns, and the representative ESTs were cloned and characterized.

POSTER PRESENTATIONS

Listed alphabetically by presenting author (**in boldface**)

Wheat chromosome group 1 mapping progress

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Wheat chromosome group 3 linkage, consensus, and synthesis maps

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Species with limited polymorphism, such as those of the Triticeae tribe, tend to have poor marker coverage of their respective genomes, making it difficult to evaluate the gap between physical and linkage distances within species. Endeavoring to facilitate this evaluation, our research group has utilized existing map data of members of the Triticeae to compile an updated consensus map for Triticeae chromosome 3. Recent efforts aiding the establishment of such a map include the reconstruction of previously existing maps and the localization of new genes. Of special interest to this research group has been the creation of a more accurate hexaploid wheat map, using the Synthetic × Opata mapping population, and the improved mapping of the locus for red kernel color.

GrainGenes: The Triticeae Genome Database

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GrainGenes, the Triticeae genome database, is the most comprehensive source of molecular and phenotypic information on wheat, barley, rye, oats, and sugarcane. Curated as a joint effort at the USDA-ARS-WRRC in Albany, CA and the USDA-ARS Center for Bioinformatics and Comparative Genomics (CBCG) at Cornell University (Ithaca, NY), GrainGenes is a central repository for information on genetic and physical maps, DNA probes, germplasm, pathology, QTLs and other accessions. GrainGenes is a publicly accessible database, administered at a new facility at Cornell (see Demeter's Genomes at http://ars-genome.cornell.edu), made available though a WWW server (http://wheat.pw.usda.gov), and can be downloaded to run as a stand-alone program on any Unix, Windows 98/NT or Macintosh computer (ftp://ars-genome.cornell.edu/pub/GrainGenes). GrainGenes may also be accessed through mirror sites at INRA, France (http://grain.jouy.inra.fr) and the UK CropNet (http://synteny.life.nott.ac.uk/). Recent additions to GrainGenes include EST sequences for 468 mapped probes (KUENZEL), a wheat microsatellite map containing 279 SSR loci (RÖDER et al.), a high density barley ALFP map with images of the mapped polymorphisms (Qi), linkage maps for *Aegilops umbellulata* (ZHANG et.al.) and durum (*Triticum turgidum*, BLANCO et al.), and an online version of the Catalogue of Gene Symbols for Wheat. Planned additions to GrainGenes in 2000 include new Triticeae consensus maps and enhanced educational facets including an interactive graphical interface of a developing wheat plant. In July, 2000 GrainGenes will unveil a new database of Expressed Sequence Tags (ESTs) from the International Triticeae EST Cooperative (ITEC) (see http://wheat.pw.usda.gov/genome) with an anticipated 40,000 sequences during the first phase and a projected 300,000 ESTs sequenced for wheat and 300,000 ESTs sequenced for barley within the next four years.

Current status of Wheat Genome Project in the US—Report on generating and processing large-scale 5' EST data

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The advancement of high-throughput automated sequencing technology has allowed single-pass sequences from random cDNA clones or ESTs to be generated in a large scale at a fairly quick pace. As a result, the availability of such a large-scale EST data has provided a powerful tool for searching and characterizing genes. Hexaploid wheat is one of the most

economically important crops in the world. Because of the large genome size in wheat the strategy of having wheat genome completely sequenced for gene discovery will not be feasible in a foreseeable future. An alternative strategy of analyzing large-scale EST data, however, can offer an unprecedented opportunity for gene discovery and functional genomics study in wheat. In the US, a large-scale, multi-investigator wheat EST project in the public sector has been initiated at USDA ARS in Albany, California with funding support from NSF. In this report, we will present methods used to generate cDNA libraries and normalized cDNA libraries, method for automated cDNA sequencing and protocol we have developed to process large-scale EST data. In this report, we will also assess the quality of the cDNA libraries, the extent of redundancy, and the effectiveness of using normalization to reduce abundant transcripts.

Development of simple sequence repeat (SSR) markers from small- and large-insert libraries in pearl millet

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Identification and characterization of drought genes

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A half-million clone BAC library of durum wheat: Progress report

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This is a progress report for the construction of bacterial artificial chromosome (BAC) library of tetraploid wheat *Triticum turgidum* var. *durum.* The genotype used for this library is a recombinant substitution line of Langdon carrying a 30 cM segment of chromosome 6BS from *T. t.* var. *dicoccoides.* This segment includes a gene for high-grain protein content identified by L. Joppa and G. Hart. DNA from this line was partially digested with restriction enzyme *Hin*dIII, subjected to double size selection, electroeluted, and cloned into the pINDIGO536 BAC vector. The library consists of approximately 500,000 clones with an average insert size of 120-kb and less than 2% empty clones. More than half of these clones have been picked and sorted in 384-well plates. The rest of the clones will be picked during year 2000, and the complete library will be organized in 27 high-density filters with 18,432 double spotted clones using a Q-bot. Excluding the empty clones the coverage of this library will be between four and five haploid genome equivalents. Plasmid DNA is being extracted from bulk cultures of each 384-well plate for PCR screening (e.g., microsatellites). For current status of the library, see: http://agronomy.ucdavis.edu /Dubcovsky/BAC-library/BAC_Langdon.htm.

Progress in mapping chromosomes of homoeologous group 4

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Use of EST-derived microsatellites to estimate genetic diversity in commercial durum wheat

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Mapping of *Pto*-like and NBS-LRR resistance gene analogs in wheat (*Triticum aestivum* L.)

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Application of comparative genetics to tef (*Eragrostis tef*) improvement

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Tef (*Eragrostis tef*) is major cereal food grain grown in Ethiopia. The goal of this study was to develop a genetic link between tef and other model crop species through the use of comparative genetic analyses. The genetic improvement of tef using mapped RFLP markers and microsatellites from rice, maize, wheat, and pearl millet will allow us to identify traits associated with lodging resistance. Increased resistance to lodging will enhance tef production and increase the inherent yield potential of tef. An F_9 interspecific recombinant inbred line of the cross Kaye Murri × *E. pilosa* (30-5) was evaluated in Ethiopia for important agronomic traits. Through phenotypic evaluation and the identification of quantitative trait loci using a comparative genetics approach, future marker assisted selection breeding in tef will be possible.

QTL and candidate gene mapping for preharvest sprouting in white wheat

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Pre-harvest sprouting (PHS) is defined as precocious germination of kernels on the plant prior to the harvest and is a result of inadequate grain dormancy. There is a strong association between red kernel color of wheat and resistance to PHS suggesting a tight linkage or functional relationship between red kernel color locus and the gene(s) responsible for grain dormancy. Nevertheless, wheat breeders have been able to develop white wheat varieties with improved resistance to PHS which indicates that additional loci influence dormancy in white wheat genotypes. PHS resistance in white wheat is inherited as a quantitative trait and is significantly influenced by genetic background, environmental conditions, and their interaction. We mapped 9 QTLs that influence PHS in a RI population derived from two whitewheat varities, Clark's Cream and NY6432-18, with different levels of resistance to PHS. We are testing additional populations in order to confirm and/or identify additional QTLs affecting PHS. We are saturating the genomic regions which contain significant QTLs with additional markers such as RFLP probes and SSRs mapped in the M6 × Opata population and comparative genomics. One of the QTLs, bcd1434 region on chromosome 1B, is a target for positional cloning because it was significant over multiple years and environments. We isolated 9 BAC clones from a *Triticum monococcum* library, obtained 2 BAC clones from a *T. tacuschii* library, and one BAC clone from a barley library, all containing the marker bcd1434. Individual BACs will be evaluated using a combination of informatics, gene expression analysis, and transformation to test the relationship between the candidate genes and resistance to PHS.

Molecular gene mapping in rye (*Secale cereale* L.)

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A genetic linkage map of rye was developed using a pooled F_2 mapping population created from a reciprocal cross of two self fertile inbred lines. In total 183 markers were mapped comprising 139 RFLPs, 19 isozyme and protein markers, 13 microsatellites, 10 known function sequences, and two morphological genes. The markers are randomly distributed via the 7 chromosomes with a maximum of 38 on chromosome 5R and a minimum of 19 on chromosome 3R. The gene loci were either directly mapped scoring the pooled F_2 population of the reciprocal cross (12 loci) or aligned to chromosome regions based on mapping data published recently (23 loci). This alignment became possible, because common markers could be used as anchors. In addition 25 quantitative trait loci were detected. For some of the mapped or aligned genes comparable loci are present in other cereals. The homoeologous relationships of these loci are discussed. The potential of the presented map for further genetic studies is outlined.

Targeted development of a multiple-allele microsatellite marker associated with a true loose smut resistance gene in barley (*Hordeum vulgare*)

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Over the past decade DNA markers have become increasingly important in plant breeding programs. PCR-based markers developed from RFLPs, RAPDs, or AFLPs are often not polymorphic in many cross combinations limiting their value. Microsatellite markers have many of the properties of an ideal marker including high levels of polymorphism, however development of microsatellite markers is tedious, time-consuming, and expensive. Although microsatellite markers have been developed in various crops, these markers are not yet widely used for marker-assisted breeding. The major challenge is that randomly developed microsatellite markers have rarely been associated with economically important traits. In the present study we report on the targeted development of a microsatellite marker to a barley disease resistance gene. The method includes the following steps: (1) pooling DNA samples from a segregating population based on the principle of bulked-segregant analysis; (2) digesting the pooled DNAs and ligating adaptors; (3) selectively amplifying and identifying polymorphic microsatellites; and (4) developing primers for the microsatellite associated with the targeted trait. Using this method, a microsatellite marker associated with the true loose smut resistance gene (*Un8*) in the Harrington × TR306 doubled haploid population was identified. This marker mapped to chromosome 1H, 7 cM from *Un8* toward the telomere. Four other breeding populations segregating for true loose smut resistance were used to evaluate the microsatellite marker. Three populations showed polymorphism. We further tested the polymorphism of the microsatellite between the resistant parent TR306 and 24 cultivars. The 24 cultivars displayed five alleles for this microsatellite and polymorphisms were found between TR306 and 18 susceptible cultivars. This novel method for the targeted development of microsatellite markers should have widespread applicability and efficiently provide highly polymorphic markers for use in breeding programs.

The structure and function of the expressed portion of the wheat genomes: The NSF-funded US wheat genomics project

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The goal is to generate and map a large number (target is 10,000) of unique DNA sequences from the genomes of bread wheat. The assumption is that these unique DNA sequences will correspond to individual genes of wheat and their identification is a first step in determining the function of these genes. The ultimate use of this information is the improvement of wheat quality, yield, and adaptability to new and marginal environments, thus increasing production.

Wheat belongs to a group of closely related species (termed a tribe, named Triticeae) in the grass family which includes more than 300 species, including several very important crops (bread and durum wheats, barley, rye, triticale) and several forage-grass species. World-wide, wheat is the most widely grown crop and the third in economic significance for the United States. The US is the largest wheat exporter in the world and, to maintain this market continuous genetic improvement of the crop is required.

Recent advances in plant genetics and genomics offer unprecedented opportunities for discovering the function of genes and potential for their manipulation for crop improvement. Because of the large size of the wheat genome (the total DNA or genetic information of the species), it is unlikely that the actual base-pair sequences of the DNA molecules will be learned completely in the near future. This project takes an alternative strategy to realize the benefits of new techniques for discovering genes and learning their function (functional genomics). Following the identification of 10,000 unique wheat DNA sequences (termed ESTs, Expressed Sequence Tags), they will be mapped to their physical location on the chromosomes of wheat. This process utilizes a unique feature of the wheat chromosomes, their ability to tolerate deletions of portions of the chromosomes and still produce a viable plant. The mapping logic is direct: if an EST is present in a plant with complete chromosomes, but absent in a plant missing

a known part of a single chromosome, then it can be inferred that the DNA sequence that corresponds to that EST is located in that segment of the chromosome.

By the end of the mapping component of this project, a most valuable tool will have been produced: 10,000 unique DNA sequences, likely corresponding to genes, whose physical location in the chromosomes of wheat are known. This sets the stage for the next phase, the analysis of this array of mapped ESTs to determine function. The project will focus on characteristics of the wheat reproductive stages, from flowering signals through seed development and dormancy. The information gathered on the sequence, function, and position of these genes in the wheat chromosomes will be collected and distributed by means of a USDA public database of genomics information (known as GrainGenes).

Because of the close relationship of wheat to other species in the Triticeae tribe and other grass species, especially corn and rice, the results from this project will be immediately applicable to other crops in the Triticeae. Most of the collaborating investigators are already collaborating members of the International Triticeae Mapping Initiative which has produced molecular genetic maps of the chromosomes of wheat and related species. The diversity of experimental techniques and traits pursued in the individual laboratories collaborating on this project will be an ideal training ground for graduate students and postdoctoral scientists. The large pool of well-characterized and mapped unique DNA sequences, available in the public domain will be an exceedingly important resource for future Triticeae research and basic functional genomics research.

Rapid EST mapping and genotyping of SNP loci using pyro-sequencing

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Single nucleotide polymorphisms (SNPs) in maize: Identification, genetic mapping, and haplotype structure

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Genotypic databases for wheat and barley based on mapped microsatellite markers

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Barley

48 mapped and highly informative barley microsatellites isolated during BBSRC PAGA and EU projects (Genotyping set 1) were selected to analyze 500 barley varieties. The microsatellites (SSRs) were selected on the basis of their i) good genome coverage, ii) high information content, and iii) high quality amplification profile. An additional, complementary set of 48 has now also been identified (Genotyping set 2). Barley varieties for this study were selected from spring and winter pedigrees, UK National and Recommended lists, and a core collection of European material.

An initial overview of raw 'Genotyper' output has confirmed the reliability of the detection system and analysis. Two out of the first 96 individuals to be analyzed (cv. Blenheim and cv. Alexis) were included in eight 'reference' genotypes used initially to test the markers in Genotyping Set 1. Allele scores taken from separate gels corresponded to within ± 0.75 bp. Over the next 6 months it is anticipated that analysis of all 500 varieties with 48 SSRs will be completed. If this can be achieved then we intend to employ additional SSRs from the complementary set of 48 (Genotyping set 2) to increase genome coverage. In addition, we intend to focus on a specific area of the genome with additional SSRs to obtain high resolution genotypic data. We believe that it will be important to ascertain whether chromosomal haplotypes derived from c. 10 markers distributed across a linkage group with an average spacing of 20cM is a good general indicator of identity by descent across the region between the markers or whether significantly more recombination has occurred but is invisible at this resolution of analysis. This will have obvious consequences for the confidence levels surrounding the future interpretation of the genotypic data.

Wheat

At the JIC the objective is to generate a comprehensive database of genotypic information of wheat cultivars currently included in European and CIMMYT breeding programmes using a set of mapped microsatellites markers. The resulting interactive database will be made available to academic and private sector scientists. To this end a collection of 225 wheat cultivars of known pedigree has been made and sufficient

DNA has been prepared from all of them for this project. Varieties were chosen to cover both CIMMYT and European breeding programmes, all relevant pedigrees, and some varieties of note from Australia and the USA. Breeding data has been assimilated from several sources including the JIC Germplasm database, CIMMYT's IWIS database, and information supplied by individual plant breeders and a comprehensive pedigree has been prepared which demonstrates the inter-relatedness of the world's wheat breeding programmes.

More than 190 mapped microsatellite loci have been selected for consideration and to date 105 have been evaluated as genotyping markers. As with barley, criteria to be satisfied include genome coverage and confidence of scoring. Sets of markers have been developed which are suitable for simultaneous analysis to optimize sample throughput and data acquisition. The robustness of the data acquisition has been ascertained by analyzing replicate samples on both ABI373 and ABI377 instruments and we are confident that both the markers and the generated data can be transferred between laboratories. Markers which produce a complex amplification pattern or which amplify from more than one locus have been discarded if they were difficult to score with confidence. Selected SSR markers developed from wheat ESTs are now being considered for inclusion with the expectation that these will prove to be useful markers for putative known-function genes. Microsatellite markers are ideal for high-throughput genotyping, their relatively high polymorphism information content and their requirement for only small amounts of DNA combined with the ability to multiplex their analysis has allowed us to collect in excess of 5000 data-points so far from the first selection of 64 related cultivars. It is anticipated that with the groundwork done—cultivar collection completed and marker sets established, the increased throughput afforded by the access to both ABI373 and ABI377 machines will allow the data collection phase of the project to be completed within six months. We will then concentrate on the development of the database and query systems.

Genes controlling the early stages of meiosis in wheat

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In planta study of meiosis-specific genes in *Triticum aestivum*

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Genes responding to Hessian fly feeding

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QTLs for grain dormancy in rice

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Seed dormancy is one of the most important traits in rice breeding because it is associated with pre-harvest sprouting (PHS) which reduces the quality of the seed. PHS is a significant problem in rice cultivated under humid environments and is quantitatively inherited. The improvement of crop species for quantitative traits is difficult because the gene(s) controlling the traits of interest are not readily detected and/or have very low heritability. Molecular marker technology is an excellent tool to study the quantitative traits of interest. In this research, molecular markers will be used to study quantitative trait loci controlling dormancy in rice. A total of 300 F_2 families derived from the interspecific cross of *Oryza sativa* ssp. *indica* (IR64) and *Oryza rufipogon*, a wild rice relative that has strong dormancy, will be analyzed by using molecular markers. Fifty seeds produced by each of the F_2 plants were tested for germination under room temperature and dark conditions. The germination rate after soaking for 12 days ranged from 0 to 100%. Forty eight F_2 families were strongly dormant, with seed germination $\leq$ 25%, and 215 F_2 families were weakly dormant, with seed germination $\geq$ 50%. The study is still underway to analyze DNA of the families using molecular markers including some SSRs and candidate genes related to dormancy from other crops such as wheat. Our goal is to study the nature and inheritance of the genes underlying PHS using comparative mapping among other cereals.