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The Barley Microarray. A Community Vision and Application to Abiotic Stress

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Abstract: A microarray chip representing approximately 20 000 barley unigenes was produced as part of a USA project entitled "An integrated physical and expression map of barley for *Triticeae* improvement". The content of the chip was derived from more than 400 000 barley "Expressed Sequence Tag" (EST) sequences received from cooperators in USA, Germany, Australia, Japan, Scotland, and Finland, plus about 1000 sequences retrieved from the GenBank nr database or GrainGenes. All EST sequences were trimmed to high quality regions, contaminants were identified and removed, and the remaining information was compiled using the CAP3 assembly program. A "stringent" assembly (paralogs separated) contained about 53 000 "unigenes" (the sum of contigs plus singletons), among which about 50% had reliable 3' ends and were therefore suitable for chip content. From these, probe sets were designed and the "Barley1" chip fabricated by Affymetrix. Complete details on the content of the barley microarray, and enhanced probe-set annotations, can be obtained using the software HarvEST:Barley, available from http://harvest.ucr.edu. The availability of an Affymetrix barley microarray has facilitated the study of gene expression on a large scale. Replicated experiments have revealed commonalities and differences between responses to abiotic stresses, and inherent differences between barley genotypes. The design and a brief summary of the results of drought stress experiments are stated.

Keywords: genomics; ESTs; microarray; abiotic stress

The "Barley1" microarray continues a tradition of cooperation and sharing within the worldwide barley community. It is my pleasure to provide some insights about the contributions of many people who participated in the development of this new resource. I will also take this opportunity to provide some simple numerical assessments of the "Barley1" array, which is far from being a "whole-genome" device, so that we do not become complacent by falsely thinking that we have the ultimate expression profiling tool. Certainly we do not, but we can and will do better in the future by continuing to work together as a community. Finally, I will briefly describe some of the experiments that my group has conducted using the "Barley1" microarray to study the transcriptional response of barley to drought stress, one of several abiotic stresses including drought, low temperature and salinity that we have studied using the Barley1 microarray.

The period of 1998 to 2002 was an incubation time for the Barley1 microarray. During this time representatives of the international barley genomics community met formally and informally at the annual Plant and Animal Genome (PAG) Conference in San Diego, California, where we exchanged information about progress and new initiatives in the development of barley genome resources, and to identify mechanisms to share them. During this period there was a consensus that a standard for parallel expression profil-

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ing in barley was needed. The diploid nature of barley, together with a tradition of cooperation in genetic resource development, gave us the sense that barley held an advantageous position as a model for Triticeae plants. This team spirit helped to drive us forward.

There was considerable discussion about what would be the most appropriate first large-scale microarray for barley. If a barley-only array were produced, then would barley become isolated from other Triticeae transcriptome research? If an all-Triticeae array (we used the term "Trit-chip") were produced from A, B, D, H, R, S and possibly other *Triticeae* genomes, then would it adequately support any single Triticeae genome, including barley? We debated whether there should be a single barley genotype, or several ones, represented on the array. If just one, then which one, my favourite or yours? If more than one, then what would be the mechanism of representing different alleles? There was also considerable debate over the appropriate format for a microarray. Should it be composed of whole cDNAs, 3' ends of cDNAs, oligonucleotides (oligos) in the 60–70 length range, or smaller oligos? Whole cDNAs would hybridise across many *Triticeae* genomes and therefore a cDNA-based chip might be most broadly useful, but precision would be compromised by cross-hybridisation between signals from paralogs within any single genome and by homoeologs in polyploid *Triticeae*. On the other extreme, multiple gene-specific 25-mers would provide excellent gene-specificity but require (at the time) some hundreds of thousands of dollars for the design fee alone. The literature of the period contained impressive research articles with data from each format. Images of thousands of green, red and yellow spots that could be scanned by the human eye with the assistance of image analysis software were popular in the late 1990's. Larger datasets and attention to statistical validity had become more prominent elements of microarray publications by 2002. Where would barley place its first community chips? The merits and limitations of each scenario were debated at PAG, every other venue that I can remember, and through waves of emails preceding various grant proposal deadlines. In retrospect, the discussion process was extremely useful as a mechanism of community education, preparing us to recognize and seize tangible opportunities. But, the decision to produce an Affymetrix chip for barley was ultimately a pragmatic choice made by a few rather than a declaration of worldwide consensus. Engagement in this plan by worldwide colleagues came naturally from our habits of collegiality. Without a doubt, this had a positive outcome on the quality of the final product.

A group of US investigators including Andris Kleinhofs (Principal Investigator, Washington State University), Gary Muehlbauer (University of Minnesota), Rod Wing (Clemson University), Roger Wise (Iowa State University) and me, received grant funds in 2001 from the United States Department of Agriculture Initiative for Future Agriculture and the Food Supply Program that allowed us to move forward. Roger Wise, in particular, deserves credit for spearheading the decision to place most of the resources from this grant on the development of a microarray and its immediate use. Within this group, we had quite some debate over chip format. Our initial plan was to use a commercial provider to spot 10 000 whole cDNAs, each representing a single "unigene". From my own perspective, as one who has an interest in a multigene family related to abiotic stress, the dehydrin gene family (Choi *et al*. 1999), whole-cDNA arrays did not appear to be a satisfactory format. Just one or a few cDNAs from this family would represent them all. There would then be no possibility to examine the expression of each gene individually. This same concern applied to resistance gene analogues and all other multigene families. I advocated against the whole-cDNA format. For a brief time, our plan shifted to the use of 3' ends of unigenes (truncated cDNA clones), but this required a different databasing method and sequencing strategy than we had initiated. Our shift of plans in favour of gene-specificity prompted Roger Wise to explore options to have commercial providers produce single 60- to 70-mer or multiple 25-mer arrays. We nearly chose the single long oligo method but in the end we were swayed by several apparent advantages of multiple short 25-mers. These advantages included: (1) considerable cost savings in chip fabrication, (2) representation of a larger number of unigenes, each with more probes per unigene, and (3) less chance of bias in signal detection by multiple oligos versus a single oligo. The latter has since been validated in the literature (for example, Rogojina *et al*. 2003).

Affymetrix (www.affymetrix.com) was the provider of the format that we chose. However, one

catch was that we had to provide our own informatics up to the point of delivery of "exemplars" (trimmed unigene sequences). The separation of paralogs and determination of orientation of unigene sequences to support the gene-specific oligo design demanded further changes in our databasing methods. Fortunately, my colleague Steve Wanamaker and I had already developed a relational database for barley and other *Triticeae* ESTs (HarvEST, http://harvest.ucr.edu) that could be readily adapted to accommodate these needs. HarvEST originated as an in-house tool for EST data management related to the design of oligos for PCR and BAC library probing. The HarvEST database became our informatics engine and subsequently provided foundational data for other viewing tools, foremost among which is BarleyBase (http://www.barleybase.org/), an excellent product of colleagues at Iowa State University.

It was an enlightening and enjoyable experience to be deeply engaged in the development of content for the Barley1 microarray (Close *et al*. 2004). It was also gratifying to turn our attention back to abiotic stress, and to see many colleagues succeeding with their use of this new resource.

MATERIAL AND METHODS

ESTs and other sequences. The first objective in the design of a microarray was to create a considerable "Expressed Sequence Tag" (EST) resource, from which chip content principally could be drawn. From January to October 2002, each project transmitted their EST data to my group at University of California, Riverside. This included either a pair of matching sequence and quality value files generated using the base-calling software "phred" from each EST (Ewing & Green 1998; http://www.phrap.org/) or the original chromatogram from which we then extracted the two phred files. By the end of 2002, worldwide projects had contributed approximately 350 000 high-quality barley ESTs originating from more than 400 000 raw sequences, constituting about 25 000 "unigenes" with satisfactory 3' ends for chip content. These came from 84 cDNA libraries representing various developmental stages, in addition to abiotic- and biotic-stress treatments (Table 1). Most of the EST data contributors and their barley EST projects are cited below.

Clemson University Genomics Institute, Clemson, South Carolina, USA: Rod Wing and Dorrie Main, with EST production support from Dilara Begum, David Frisch, Michael Atkins, Yeisoo Yu, D. Henry, M. Palmer, T. Rambo, J. Simmons and R. Oates. Supported by the United States Department of Agriculture – National Research Initiative project, "Establishment of a Genetically and Physically Anchored EST Resource for Barley Genomics" (Andris Kleinhofs, PI; Rod Wing, Timothy Close, Roger Wise).

Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany: Andreas Graner, Nils Stein and Winfriede Weschke with support from Hangning Zhang, Elena Potokina, Volodya Radchuck and Jelena Perovic. Barley ESTs from the project "Partial Sequencing of cDNAs for the Generation of Expressed Sequence Tags (ESTS) of Barley" funded by the state of Saxony-Anhalt. Libraries and funding for these ESTs were also provided by the project "An Expressed Sequence Tagged (EST) database of barley" (Andreas Graner and Peter Landrigde PIs), funded by the Grains Research and Development Corporation, Government of Australia.

Okayama University, Japan: Kazuhiro Sato, Daisuke Saisho and Kazuyoshi Takeda. National Institute of Genetics, Japan: Yuji Kohara and Tadasu Shin-i. ESTs from the collaborative project between the Barley Germplasm Centre, Okayama University and Centre for Genetic Resource Information, National Institute of Genetics. This project has been supported by a Grant-in-aid for Scientific Research on Priority Areas C from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan and by CREST (Core Research for Evolutional Science and Technology) of JST (Japan Science and Technology Corporation).

Scottish Crop Research Institute (SCRI), Invergowrie, Dundee, Scotland, UK: Robbie Waugh, Peter Hedley, H. Liu, D. Caldwell, Luke Ramsay, David Marshall, and Linda Cardle. Developed as part of the barley transcriptome resources of BBSRC/SEERAD funded by Cereal Investigating Gene Function project.

Hans Bohnert (USA), Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, and Plant Sciences and Department of Crop Sciences, University of Illinois, Urbana-Champaign. Supported by NSF – Plant Genome Program (98-13360); data collected by: N. Z. Ozturk, C. B. Michalowski, S. Brazille, C. Borchert, C. Palacio, C. Normand, C. Murphy,

R. Kelley, S. A. Sant, H. McLaughlin, and M. A. Fredricksen.

Institute of Biotechnology, University of Helsinki, Helsinki, Finland and MTT Agrifood Research, Jokioinen, Finland: Alan Schulman, Ari-Matti Sarén, Jaakko Tanskanen, and Lars Paulin with support from Tanja Horko and Ursula Lönnqvist. ESTs from the project "Production of EST tools for barley gene discovery and exploitation" funded by major contributions of TEKES, the National Technology Agency of Finland, and Boreal Plant Breeding Ltd., as well as by contributions from Polttimo Companies Ltd., and the Raisio Group, Ltd. Additional in-kind contributions from CSC-Scientific Computing Ltd., and Visipoint OY.

In addition, Dan Ashlock at Iowa State University extracted complete barley cDNA and gene sequences from the National Center for Biotechnology "nr" database, and David Matthews, head curator of GrainGenes at Cornell University, provided sequences from Gottfried Kuenzel (IPK Gatersleben) that were available only from the GrainGenes database (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi). The final sequences represented on the chip included commonly used transgenes suggested by Peggy Lemaux and Shibo Zhang, University of California, Berkeley and several disease resistance genes and additional control sequences provided by Roger Wise, Stacey Turner and Rico Caldo at Iowa State University and by Andris Kleinhofs.

Several barley genotypes were the source of tissues for cDNA libraries and ESTs (Table 1). In general, each group chose a spring malting barley popular in their own country as the main source of ESTs. Most of the US libraries were from Morex, the German from Barke, the Japanese from Haruna Nijo, the Scottish from Optic, and the Finnish from Saana. A few other genotypes also contributed to the EST dataset, including: Golden Promise, a two-row spring barley popular for transformation; Kympii, a Finnish two-row spring malting barley that was a convenient source of callus tissue; one wild barley accession *H. vulgare* ssp. *spontaneum* OUH602; Akashinriki, another Japanese barley; Tokak, a Turkish barley chosen for drought studies (Ozturk *et al*. 2002); Rolfi, a Finnish spring feed barley studied for net blotch disease; and other genotypes containing resistance determinants in essentially a Morex or Ingrid background. The tissues spanned the range from roots to leaves to highly specialized reproductive structures. Stages of development covered the gamut from germinated seed to mature spike. Treatments included abiotic stresses and pathogen challenge in addition to normal growth. Further information on source materials is displayed within HarvEST:Barley and in GenBank accessions for each EST.

Sequence processing. Briefly, sequence processing steps (Close *et al*. 2004) were: (1) phred was applied to chromatograms to produce sequence and quality files, (2) cross match (http://www.phrap.org/) was used to mask cloning system sequences, (3) an in-house script "qvtrim" was used to synchronously remove low quality regions outside of a sliding window with an average phred quality value of 17, reduce poly(T) or poly(A) ends to a maximum of 17 consecutive T's or A's, and remove residual cloning system sequences, (4) sequences less than 100 bases after steps 1–3 were discarded, (5) a filter based on the frequency of four-nucleotide repeats was applied to remove additional ESTs that resulted from poor quality sequencing reactions, (6) orientations were determined using information on sequencing primer, high BLASTX orientation (default parameters), and presence of a poly(A) or poly(T), (7) BLASTN searches (Altschul *et al*. 1997; http://www.ncbi.nlm.nih.gov/BLAST/) were performed to identify contaminant sequences from *E. coli*, bacteriophage *lambda*, fungal sources, rRNA or the repetitive portion of *Triticeae* genomes (TREP; http://wheat.pw.usda.gov/ggpages/ITMI/Repeats/index.shtml), (8) several chimera filters, including searches for interior sequences from the cloning system or ESTs that both begin with poly(T) and end with poly(A), were applied to individual EST sequences, (9) assemblies were produced using "tgicl" from Geo Pertea at TIGR (http://www.tigr.org/tdb/tgi/software/) to manage the preclustering of ESTs using "megablast" (Zhang *et al*. 2000) and final clustering using a special version of CAP3 (Huang & Madan 1999) kindly provided by Xioaqiu Huang at Iowa State University, (10) contig orientations were determined using the ratio of forward and reverse EST sequences and the orientation of each EST used by CAP3, (11) additional chimera filters, including searches for contigs whose overall orientation could not be resolved or whose consensus sequence both begins with poly(T) and ends with poly(A), were applied to assembled ESTs, (12) assembly and chimera removal was repeated several times,

Table 1. cDNA libraries and ESTs

Library	Genotype	Source	Tissue	Stage	Condition	Clones	ESTs	% unique[a]
BaAK	Akashinriki	Japan	leaf	vegetative		10 671	19 912	7.8
BaAL	Haruna Nijo	Japan	leaf	heading		9 720	16 013	6.7
BaGS	Haruna Nijo	Japan	shoot	seedling 5 d		10 212	18 069	9.2
BaH	*spont.* OUH602	Japan	leaf	heading		13 132	23 752	14.1
BaSD	Haruna Nijo	Japan	leaf	seedling 2 wk		5 081	8 624	11.0
EBan01	Optic	Scotland	anther	pre-anthesis yellow		1 480	1 778	21.8
EBca01	Optic	Scotland	carpel	pre-anthesis		1 415	1 415	7.8
EBed01	Optic	Scotland	endosperm	6 DPA		661	661	8.0
EBed02	Optic	Scotland	endosperm	8 DPA		646	646	7.4
EBed07	Optic	Scotland	endosperm	28 DPA		1 071	1 071	3.2
EBem04	Optic	Scotland	embryo	12 DPA		1 152	1 493	5.2
EBem05	Optic	Scotland	embryo	14 DPA		1 347	1 347	7.7
EBem06	Optic	Scotland	embryo	21 DPA		1 198	1 198	5.3
EBem07	Optic	Scotland	embryo	28 DPA		1 191	1 191	5.3
EBem08	Optic	Scotland	embryo	40 DPA		1 173	1 254	5.6
EBem09	Optic	Scotland	embryo	malted 1 d		1 912	1 912	8.2
EBem10	Optic	Scotland	embryo	malted 2 d		1 327	1 345	7.6
EBes01	Optic	Scotland	embryo sac	4–6 DPA		1 283	1 283	8.3
EBma01	Optic	Scotland	maternal	4 DPA		1 047	1 047	7.4
EBma03	Optic	Scotland	maternal	8 DPA		963	963	7.4
EBma04	Optic	Scotland	maternal	10 DPA		767	767	4.6
EBma05	Optic	Scotland	maternal	12 DPA		879	879	4.6
EBma07	Optic	Scotland	maternal	21 DPA		270	270	3.3
EBma08	Optic	Scotland	maternal	28 DPA		1 159	1 159	4.5
EBpi01	Optic	Scotland	pistil	1 DPA		1 447	1 447	7.5
EBpi03	Optic	Scotland	pistil	4 DPA		1 040	1 040	6.5
EBpi05	Optic	Scotland	pistil	8 DPA		1 227	1 227	5.8
EBpi07	Optic	Scotland	pistil	12 DPA		631	631	7.6
EBro01	Optic	Scotland	root	seedling 3 wk		1 403	1 403	12.8
EBro02	Optic	Scotland	root	seedling 3 wk	low N	2 055	2 055	12.6
EBro03	Optic	Scotland	root	seedling 3 wk	water-log	2 462	2 462	9.9
EBro04	Optic	Scotland	root	seedling 3 wk	salt	1 184	1 308	11.4
EBro05	Optic	Scotland	root & shoot	seedling 3 wk	etiolated	106	106	5.7
EBro06	Optic	Scotland	root	seedling 3 wk	drought	125	125	16.0
EBro07	Optic	Scotland	root & shoot	seedling 3 wk	etiolated	822	822	5.1
EBro08	Optic	Scotland	root	seedling 3 wk	drought	3 759	3 759	17.3
HB	Tokak	US (Bohnert)	leaf	seedling 3 wk	drought	523	523	9.0
HC	Tokak	US (Bohnert)	root	seedling 3 wk	drought	928	928	23.2
HV_CEa	CI16155	US (Wing)	leaf	seedling 8 d	*Blumeria*	4 313	5 515	15.0
HV_CEb	CI16151	US (Wing)	leaf	seedling 8 d	*Blumeria*	4 298	5 997	17.1
HVSMEa	Morex	US (Wing)	shoot	seedling 7 d	cold	4 266	6 587	16.9
HVSMEb	Morex	US (Wing)	shoot	seedling 6 d	drought	4 712	6 838	18.2
HVSMEc	Morex	US (Wing)	shoot	seedling 5 d	etiolated	2 322	3 139	29.0
HVSMEf	Morex	US (Wing)	root	seedling 5 d	etiolated	5 089	7 271	22.2

Table 1 to be continued

Library	Genotype	Source	Tissue	Stage	Condition	Clones	ESTs	% unique[a]
HVSMEg	Morex	US (Wing)	spike	pre-anthesis		4 786	7 455	17.2
HVSMEh	Morex	US (Wing)	spike	5–45 DAP		4 630	5 164	8.3
HVSMEi	Morex	US (Wing)	spike	20 DAP		4 707	6 214	14.4
HVSMEk	Morex	US (Wing)	testa/pericarp	seed		4 248	6 566	16.0
HVSMEl	Morex	US (Wing)	spike	spike devel.	*Fusarium*	4 515	6 287	18.1
HVSMEm	Morex	US (Wing)	leaf	seedling 8–10 d	*Blumeria*	4 491	6 284	24.9
HVSMEn	Morex	US (Wing)	rachis	developing		4 321	6 152	28.6
IPK_HA	Barke	Germany	embryo sac	0–7 DAP		9 944	11 270	7.4
IPK_HB	Barke	Germany	caryopsis	8–15 DAP		10 779	10 987	8.7
IPK_HD	Golden Promise	Germany	embryo	callus		4 659	5 361	15.1
IPK_HE	Barke	Germany	leaf	seedling 7 d	etiolated	312	511	6.7
IPK_HF	Barke	Germany	caryopsis	16–25 DAP		7 489	7 756	8.2
IPK_HG	Barke	Germany	leaf	seedling 7 d		324	535	3.4
IPK_HI	Barke	Germany	fem. inflorescence	pre-anthesis 3 mm		4 256	4 936	10.7
IPK_HK	Barke	Germany	leaf	seedling 6 d	etiolated	1 050	1 436	17.8
IPK_HM	Barke	Germany	male inflorescence	pre-anthesis 2 mm		4 204	4 858	14.5
IPK_HO	Ingrid BC mlo5	Germany	epidermis	seedling 7 d	*Blumeria*	4 796	4 798	22.5
IPK_HP	Barke	Germany	epidermis	seedling 7 d		236	406	14.4
IPK_HR	Barke	Germany	root	seedling 2–3 d		330	534	12.1
IPK_HS	Barke	Germany	embryo	malted 0–16 h		4 630	7 238	16.3
IPK_HT	Barke	Germany	endosperm	malted 0–16 h		4 920	7 248	16.3
IPK_HU	Barke	Germany	seed	malted 16–48h		4 609	7 313	17.6
IPK_HV	Barke	Germany	seed	malted 48–96 h		4 493	7 122	16.2
IPK_HW	Barke	Germany	root	seedling 2 d		3 165	5 960	19.3
IPK_HX	Barke	Germany	apex 3-5 mm	adult		4 876	5 681	9.9
IPK_HY	Barke	Germany	caryopsis	3–29 DAP		3 480	6 298	11.6
IPK_HZ	Barke	Germany	pericarp	0–7 DAP		10 067	10 072	8.9
S00002	Saana	Finland	embryo	1 DAP		5 094	5 263	8.2
S00007	Saana	Finland	shoot	2, 3, 4 d		3 461	3 842	8.7
S00008	Kymppi	Finland	callus K19	callus		9 903	10 269	11.3
S00010	Saana	Finland	seed	1–9 DAP		1 444	1 444	9.2
S00011	Saana	Finland	seed	12–18 DAP		10 401	10 904	4.4
S00014	Rolfi	Finland	leaf	seedling 2 leaf	*Pyrenophora*	842	875	5.5
S0MISC	Saana	Finland	various	various		224	224	8.9
WHOLE	Genbank nr	US (Wise)	various	various	various	977	977	15.0
KUENZEL	Kuenzel probes	US (Matthews)	various	various		22	22	4.5
CPLAST	Chloroplast	various	various	various	various	44	44	100.0
MITO	Mitochondrion	various	various	various	various	44	44	100.0
BARLEY1X	Other	US (Wise/ Kleinhofs)	various	various	various	127	127	100.0
Totals						265 383	349 709	

[a] % unique is defined as the number of contigs and singletons that are unique to the library divided by the number of clones sequenced from the same library

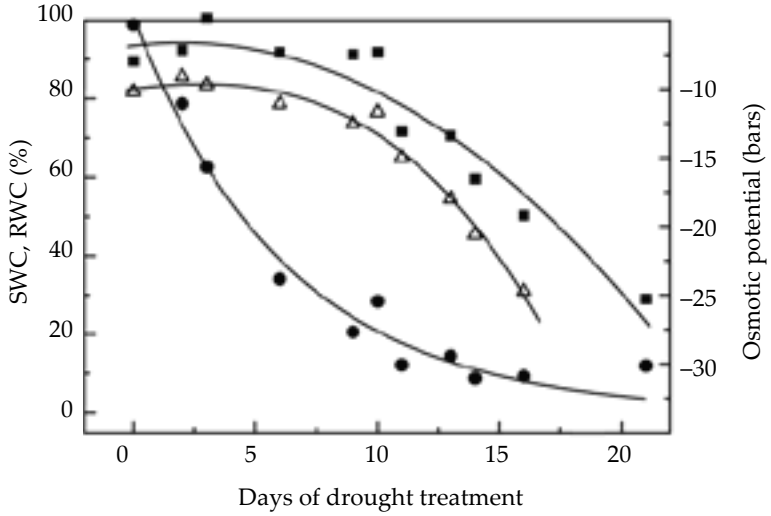
(13) sequences with reliable 3' ends were determined, (14) reverse orientation unigene sequences were converted to forward-oriented sequences, (15) multiple poly(A) sites were trimmed to their first poly(A) site to define "exemplars" that were transmitted to Affymetrix for probe set (oligo) design, (16) several unigenes were designated as standards for labelling controls, (17) additional sequences were added as controls and for other anticipated research purposes. All information from these steps was recorded in a Visual FoxPro relational database, from which the HarvEST: Barley software is an extraction product. Annotations of the probe sets (rice gene model, etc.) can be exported from HarvEST:Barley using the "Search the Barley Chip" function.

Drought stress. Morex barley seeds were sown in pots filled with a standard soil mixture at a density of 40 seeds per pot. Plants were grown in a growth chamber with 23/20°C day/night temperatures and 12 h photoperiod. Ten days after sowing, water was withheld from "stressed" material, while watering was continued for unstressed reference material. The pots were weighed at regular intervals for calculation of the soil water content (SWC). SWC was determined as the water content relative to the total soil water content 16 h after complete hydration. For each of three fully replicated experiments, samples were taken when the SWC was approximately 70, 35, 20, 12 and 8%. Leaf relative water content (RWC) was measured in two fully expanded leaves. Osmotic potential was measured in pressure-extruded leaf-sap using a vapour pressure osmometer (model 5100C, Wescor Inc. Logan, Utah) with sugar solutions as a calibration curve. Experiments were also conducted on low temperature, salinity and abscisic acid treatment (not shown). Total RNA was isolated from crown tissue using TRIzol Reagent following the procedure described in the Arabidopsis consortium web site (http://www.arabidopsis.org/info/2010_projects/comp_proj/AFGC/RevisedAFGC/site2RnaL.htm). Further purification was achieved using an RNeasy spin column (Qiagen, Chatsworth, CA) with on-column DNase treatment, following the manufacturer's instructions, to remove contaminating DNA and tRNAs, 5S RNA and most RNA less than 200 bases.

RESULTS AND DISCUSSION

Barley unigenes and microarray content

A previous description of the content and performance of the Barley1 microarray summarized categories of exemplar sequences and data related to error rates and applicability of the Barley1 microarray to other cereal plants (Close *et al*. 2004). We stated in that publication that the Barley1 chip represents more than 21 000 non-redundant exemplar sequences. While this is correct in the context of the sequence assembly that we used, a more conservative estimate of the number of barley genes represented by the Barley1 chip is about 14 000, or about 30% of the barley genome if we assume that barley and rice have about the same number of genes. A BLASTX search of rice coding sequences available from TIGR (ftp://ftp.tigr.org/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_2.0/all_chrs/all.cds) revealed that 85.5% of 21 350 unigenes repre-



SWC – circles; RWC – squares; leaf sap osmotic potential, triangles

Figure 1. Time course of drought stress

sented by Barley 1 probe sets (18 523) match a rice coding sequence at an e-score of -5 or better, but only 11 805 rice coding sequences account for all of these matches. If we assume that the remaining 14.5% of barley unigenes (those without a high match to rice) have a similar compression rate (11 805/18 523), then the Barley1 chip may represent about 13 200 barley genes. This would be an overly conservative estimate, however, since the rice genome has not yet been fully annotated and the number of barley unigenes that match rice genes is higher if the e-score threshold is relaxed. In addition, alternative polyadenylation sites and other splice variants are often represented by the Barley1 probe sets. A conservative estimate of the number of barley genes represented on the Barley1 microarray is therefore about 14 000. From the perspective of abiotic stress, it is interesting to make an equivalent assessment considering 17 "osmotic stress related" libraries including drought, low temperature, salinity, embryo dehydration and pollen maturation. Together, these 17 libraries contributed 8 068 non-redundant unigenes (of about 21 400), among which 89.9% (7 254) match rice version 2.0 coding sequences at an e-value of -5 or better. Furthermore, these stress-related libraries account for 51.4% (6072/11 805) of rice coding sequences identified as highest BLASTX match with barley genes represented on the chip. The Barley1 microarray therefore seems to be an excellent tool for initial investigations of abiotic stress responses.

Drought stress

In the experiments described in Materials and Methods and illustrated in Figure 1, it was observed (Edmundo Rodriguez, University of California, Riverside, unpublished) that there were clear, reproducible differences in the categories of genes that were expressed at different stages of the stress treatment. For example the dehydrin genes, which are a favourite topic in my laboratory (Choi *et al*. 1999), were expressed mainly during the most severe stages of the stress treatment. In contrast, changes in the expression of transcription factors were a prominent theme of the early stage of the stress treatment. The location of some of these genes on the barley linkage map, and on the rice linkage map using orthology and synteny relationships between barley and rice, provide some intriguing examples of stress-regulated genes that are candidates for stress tolerance traits. For example, the drought-up-regulated dehydrin gene *Dhn6* is located within a region of chromosome 4H recently associated by SSR markers with drought tolerance (Ivandic *et al*. 2003).

CONCLUSION

The Barley1 microarray provides an excellent starting point for global analysis of gene expression in abiotic stress and other aspects of barley, representing some 30% of the genes in the barley genome. Major changes in transcriptional activity can readily be measured using this new device, and the standardised platform facilitates data sharing. It is now appropriate for the barley community to engage in the design of a microarray representing a larger number of barley genes.

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Abstrakt

Close T. J. (2005): **DNA pole ječmene. Společná vize a aplikace na studium abiotických stresů**. Czech J. Genet. Plant Breed., **41**: 144–152.

DNA čip (microarray) reprezentující přibližně 20 000 genů ječmene byl vytvořen v USA jako část projektu, který má název „ Integrovaná fyzikální a expresní mapa ječmene pro šlechtění *Triticeae*". Obsah čipu byl odvozen z více než 400 000 EST (Express Sequence Tag) získaných od spolupracovníků v USA, Německu, Austrálie, Japonsku, Skotsku a Finsku a 1000 sekvencí obdržených z databází GenBank nr nebo GrainGenes. Všechny EST sekvence byly vybrány na základě vysoké kvality, příměsi byly identifikovány a odstraněny, a zbývající informace byla zkompletována s využitím programu CAP3. Stringentní soubor (s odstraněnými „paralogy" sekvencí) obsahoval okolo 53 000 „unigenů" (suma „contigů a singletonů"), z nichž 50 % mělo spolehlivé 3' konce a byly proto vhodné pro obsah čipu. Z této sestavy byl navržen zkušební „Barley 1" čip, vyrobený firmou Affymetrix. Kompletní detaily, pokud jde o obsah DNA čipu ječmene, včetně rozšířených anotací použitých sond mohou být získány pomocí softwaru HarvEST:Barley, který je dostupný z http://harvest.urc.edu. Dostupnost DNA čipu ječmene firmy Affymetrix umožnila studium exprese genů ve velkém rozsahu. Opakované pokusy odhalily shodu i rozdíly v reakcích na abiotické stresy a dědičně podmíněné rozdíly mezi genotypy ječmene. V práci je uvedeno pokusné uspořádání, jakož i stručné výsledky pokusů zaměřených na studium vlivu stresu sucha.

Klíčová slova: genomika; ESTS; microarray; abiotický stres

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