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## P262

### The glutaredoxin gene family in wheat functions beyond redox homeostasis regulation

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Glutaredoxins (GRXs) are glutathione dependant oxidoreductases which mediate the reversible reduction of disulphide bonds of proteins and protein-glutathione mixed disulphides and are found in all free-living organisms. The reported biological role of GRX is in protein redox homeostasis, oxidative stress response and redox-dependant signal transduction. There are three GRX subgroups in plants, each characterised by the active site motif: CPYC, CGFS and the CC-type. There are 28 GRXs in rice indicating that there could be over 150 GRXs in wheat. Blast searches of an EST database revealed 104 expressed GRXs and several expressed pseudogenes, making the GRX family in wheat the largest discovered so far. Previous work in *Arabidopsis* has shown that the expression of CPYC and CGFS type GRXs is ubiquitous but the expression of CC type genes is indicative of roles in development and stress response. A survey of the 544 wheat GRX ESTs revealed that CPYC genes account for 59% of GRX expression, CGFS for 25% and CC-types for only 15% on the basis of EST proportions indicating that CC-type GRXs are on average expressed to a lesser degree than the “classical” GRX counterparts. The wheat database contained a partial sequence from a CC-type GRX similar to *ROXY1*, from *Arabidopsis* which is important in development of floral structures. Gene isolation work resulted in the identification three distinct *ROXY-like* genes present in wheat, which were all shown to be expressed in wheat inflorescences. The three wheat *ROXY-like* genes were all able to fully complement the *roxy1* mutation in *Arabidopsis*, implicating these three genes in inflorescence development in wheat.

## P263

### A molecular marker closely linked to the male sterile *Ms2* gene in common wheat (*Triticum aestivum*)

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A male sterile wheat mutant named ‘Taigu’ was found in a wheat field in China in 1972. The male sterility is controlled by a single dominant gene. In 1986, the male sterility gene was referred to as *Ms2*. Recently this gene was linked to a dwarfing gene through crossing ‘Taigu’ with the short wheat ‘Aibian 1’ carrying the dwarfing gene *Rht-D1c*. It has been used as a tool for backcrossing and recurrent selection in wheat breeding programs ever since. The objective of this study was to develop molecular markers linked to the male sterility *Ms2* gene in common wheat. The dwarf male sterile wheat was crossed with an adapted wheat cultivar ‘Longmai 29’ in Heilongjiang Academy of Agricultural Sciences, China. The male sterile plants of the F1 were selected and backcrossed to ‘Longmai 29’. This was repeated until BC4. An additional backcross was made with the same recurrent parent ‘Longmai 29’ in Eastern Cereal and Oilseed Research Center, Ottawa, Canada in 2002. Four sib inter-crosses were made between male sterile plants and fertile plants in each generation segregating for fertile and sterile plants. Thus, one hundred and twenty two near isogenic lines were developed through backcrossing and sib inter-crossing, and used for the development of molecular markers. In the SSR analysis, after screening 48 pairs of SSR primers, a marker, *ms2-wmc617*, was identified closely linked to the male sterile *Ms2* gene and mapped at the distal position of chromosome 4DS. The use of the molecular marker *ms2-wmc617* can facilitate recurrent selection

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in a wheat breeding program. In addition, the marker is at the same locus as the dwarfing gene *Rht-D1c*. Thus, the identification of the molecular marker, followed by the development of a fine genetic map of chromosome 4DS, could provide a firm foundation for cloning both *Ms2* and *Rht-D1c* genes.

## **P264**

### **MIKC type genes of the MADS-box family in wheat: molecular and phylogenetic analysis**

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The present paper reports the cloning and characterization of 45 full-length cDNA sequences of MIKC-type MADS-box genes of bread wheat. EST databases of wheat were searched by known sequences of MIKC-type genes and specific primers were designed for cDNA cloning by RT-PCR. Full-length cDNAs were obtained by 5' and 3' RACE extension. Southern analysis of genomic DNA detected three fragments, corresponding to the three homoeologous genes, for each of the MIKC cDNAs. Further evidence of this organization was obtained by aneuploid analysis of six *SEP*-like genes; for each of them three copies were located in different homoeologous chromosomes. The chromosome arm locations of the six *SEP*-like genes of wheat were compatible with the map locations of their orthologous genes of rice and maize. In fact syntenic relationships, and sometimes high collinearities, have been shown between the chromosomal regions where the *SEP*-like genes of rice and maize are located and the 4, 5 and 7 chromosome homoeologous groups wherein the wheat genes were located. Phylogenetic analysis assigned the wheat MIKC cDNAs to 11 of the 13 MIKC subclasses identified in plants, which correspond to most genes controlling the floral homeotic functions. The expression patterns of cDNAs assigned to different homeotic classes were analysed by RT-PCR, real time RT-PCR and northern hybridisation in 18 wheat tissues and floral organs. The potential functions of the genes corresponding to the cloned cDNAs were predicted on the basis of sequence homology and similar expression pattern with characterized MADS-box genes of *Arabidopsis* and other monocot species. The effects of thermo- and photo-period on the expression of some involved MADS-box genes was studied in two wheat cultivars with different heading time (Chinese Spring and Pandas). The expression analyses was carried out by real time RT-PCR of RNA from wheat plants exposed to different conditions of thermo- and photo-period.

## **P265**

### **Genetic mapping of *Vrn-D4* gene in hexaploid wheat**

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Natural variation in vernalization requirement in wheat is mainly controlled by four loci, *Vrn-1*, *Vrn-2*, *Vrn-3*, and *Vrn-4*. The first three have been already cloned and a model explaining their epistatic interactions has been proposed. To expand this model, we have initiated the construction of a high-density map of *Vrn-4* with the long term objective of positionally clone the gene. Natural variation for *Vrn-4* has been detected only in the D genome of wheat (*Vrn-D4*; formerly known as *Vrn4* or *Vrn-D5*). The genetic stock for the dominant *Vrn-D4* allele is Triple Dirk F (TDF, hereafter), but there has been some controversy around the *vrn-1* alleles present in TDF. Therefore, we analysed TDF seed stocks from Japan and the US using molecular markers for known *Vrn* genes. The TDF stock from Japan showed recessive alleles for all three homoeologous *vrn-1* loci and segregated only for *Vrn-D4*. On the

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contrary, the US TDF stock (from WSU) showed dominant *Vrn-A1* and *Vrn-B1* alleles, and differed from the TDF stock from Japan for the SSR markers present in the *Vrn-D4* region. These differences may explain previous inconsistencies. We crossed the TDF stock from Japan with the winter cultivar Hayakomugi (*vrn-D4*, Japan) and generated a mapping population of 258 F<sub>2</sub> plants. Unvernalized plants grown under long day conditions (16-h light) showed a 3: 1 ratio between spring and winter lines, confirming that *Vrn-D4* was the only growth habit gene segregating in this population. *Vrn-D4* was mapped on the centromeric region of chromosome 5D completely linked to SSR locus *Xcdf67*, and was flanked by *Xcdf81* (8.4 cM) on the short arm and *Xbarc205* (1.1 cM) on the long arm. The arm location of *Vrn-D4* is still unknown. We are currently adding SNPs markers to the region to generate a comparative map with rice and to saturate the *Vrn-D4* region with additional markers. Simultaneously, we are increasing the size of our mapping population.

## **P266**

### **Physical map of the *Eps-A<sup>m1</sup>* gene region in *Triticum monococcum* L.**

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High grain yields in wheat require a precise optimization of resource allocation during its life cycle and, therefore, the timing of each developmental phase is extremely important. Earliness *per se* (*Eps*) genes are responsible for the fine-tuning of flowering time independently of vernalization and photoperiod, the main environmental signals controlling the initiation of reproductive development in plants. We mapped an *Eps* gene, designated *Eps-A<sup>m1</sup>*, in the distal region of chromosome 1A<sup>mL</sup> in cultivated diploid wheat (*T. monococcum* L.). We constructed a high-density genetic map of the *Eps-A<sup>m1</sup>* region using a BC<sub>6</sub>F<sub>2</sub> population of 8,788 near isogenic lines (NILs) segregating only for the *Eps-A<sup>m1</sup>* region. We saturated the region with molecular markers derived from the rice colinear region and from *Brachypodium*, *Ae. tauschii*, and *T. monococcum* BAC clones identified during the chromosome walk. We mapped the *Eps-A<sup>m1</sup>* gene within a 0.6-cM region flanked by genes *Cf2* and *Adk1*. Using *Adk1* as a starting point we constructed a complete physical map of the *Eps-A<sup>m1</sup>* region in *T. monococcum* including five overlapping wheat BAC clones covering a 400-kb region. Partial sequencing of this region revealed seven candidate genes completely linked to *Eps-A<sup>m1</sup>* (*FtsH*, *Snf1*, *Fop1*, *Ulp1*, *Akl1*, *Akl2*, *Dhh1*). Using NILs with recombination events close to the *Eps-A<sup>m1</sup>* gene, we found that the late flowering of the lines carrying the allele from cultivated *T. monococcum* could be attributed to both a delay in the transition between the vegetative and reproductive apices and an extended spike development phase. This extension was associated with a 32% increase in the number of spikelets per spike, relative to the lines carrying the allele from wild *T. monococcum*. Chinese Spring (CS) lines lacking the complete chromosome 1A, its long arm, or the distal region of its long arm also flowered earlier and had a smaller number of spikelets per spike than CS, suggesting that a gene similar to *Eps-A<sup>m1</sup>* is present in the colinear region of hexaploid wheat.

## **P267**

### **Genetic analysis of quantitative traits in wheat (*Triticum aestivum*)**

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Grain yield is a complex trait made up of the interaction between different yield components and environmental effects. Because of these complex interactions it is difficult to improve yield through breeding (especially in the early generations) if yield is the only factor recorded, suggesting that

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component traits should also be used as selection criteria for yield improvement. This is the reason why it is necessary to know the genetic architecture of yield components. The objective of this investigation was the study of inheritance of the quantitative traits in bread wheat by using generations mean analysis (GMA). Two inbred lines (falat & line 30) were crossed to produce the F1 generation. The resulting F1 plants were selfed and backcrossed to both parents to produce F2 and backcross generations respectively. All generations (Parents, F1, F2, BC1 and BC2) were planted in agricultural faculty field of Tehran university using a Randomized Complete Block Design with three replications. GMA was performed by scaling test which assays all generations simultaneously. In most cases a digenic epistatic model was sufficient to explain variation in generation means. Gene effects including mean effect, additive, dominance, epistasis effects of additive  $\times$  additive, additive  $\times$  dominance and dominance  $\times$  dominance were observed. For plant height and 1000 seed weight the additive- dominance effects and for other traits the epistasis effects (Specially additive  $\times$  additive and dominance  $\times$  dominance) were distinguished the most important effects in controlling the heritability of these traits. The highest (68%) and lowest (12%) narrow sense heritability were obtained for kernel wt./plant and peduncle out, respectively.

Key words: Generation mean analysis, bread wheat (*Triticum aestivum*), Genetic analysis, quantitative traits, Gene effects.

**P268**

**Assaying natural genetic variation in the cereal *VRNI* gene**

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The *VRNI* gene is the major determinant of the vernalization requirement in barley and wheat. Deletions in the first intron of *VRNI* cause increased *VRNI* activity in the absence of cold treatment and reduce the vernalization requirement. We assayed the extent of natural variation in the first intron of the *VRNI* gene amongst four thousand barleys and three thousand wheats from diverse geographical regions. Ten alleles of the barley *VRNI* gene (*HvVRNI*) were identified, including some alleles that have not been described previously. Different *HvVRNI* alleles were often associated with specific geographical regions. Similarly, new alleles of *TaVRNI* were identified in wheat, and an association between *TaVRNI* genotypes and geographical regions was also observed. Comparison of the position and length of deletions in the first intron of *HvVRNI* have allowed us to identify regions required for repression of this gene prior to winter. We also found a correlation between the length of deletions in the first intron and *HvVRNI* expression levels. Deletion of the critical regions within the *HvVRNI* intron does not prevent induction of *HvVRNI* by low temperatures. We suggest that regions within the first intron of *HvVRNI* are required to maintain low levels of *HvVRNI* expression prior to winter but act independently of the regulatory mechanisms that allow low-temperature induction of *HvVRNI* during winter.

**P269**

**Isolation of a somatic embryogenesis receptor kinase gene from wheat and assessment of its role in transformation**

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Increased shoot regeneration from *in vitro* culture via overexpression of somatic embryogenesis receptor-like kinases (SERKs) have been reported in *Arabidopsis* and *Oryza sativa*. In order to

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determine whether a similar increase may occur in *Triticum aestivum* a cDNA clone with an open reading frame encoding a protein with high homology to SERK proteins was identified using a wheat EST database. The sequence of this SERK more closely resembled *Zea mays* SERK2 than *Zea mays* SERK1 or *Oryza sativa* SERK1 and was designated TaSERK2. Use of the *TaSERK2* gene in a wheat transformation protocol did not result in enhanced transformation efficiency and transgenic plants expressing *TaSERK2* constitutively did not exhibit enhanced shoot regeneration capacity. However, use of an RNAi vector designed to knockout *TaSERK2* expression resulted in a reduction in transformation efficiency and plants expressing the RNAi construct did not produce T1 progeny carrying the construct. This indicates that expression of *SERK* is necessary for efficient transformation but that another *SERK* gene other than *TaSERK2* is the critical element. It is possible that the critical SERK protein may be one with greater homology to SERK1 types and there is evidence from the expressed sequence tag database that such a protein exists in wheat.

**P270**

**General disease resistance loci against biotrophic pathogens in wheat**

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There is increased interest in breeding wheat cultivars for partial and race non-specific resistance against diseases like leaf rust (LR), stripe rust (YR) and powdery mildew (PM) which are caused by biotrophic pathogens with high evolutionary potential. The CIMMYT bread wheat line Saar exhibits good partial resistance to all three pathogens, and a QTL mapping study was conducted in a cross with the susceptible line Avocet-*YrA*. The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* were shown to be major determinants of the PM resistance in Saar as well as conferring resistance to LR and YR. The PM resistance genes at these two loci on chromosomes 7DS and 1BL have been named *Pm38* and *Pm39*, respectively. Further characterization of the resistance was conducted with near-isogenic lines in the susceptible background of Avocet-*YrA*. Both loci were shown to confer partial resistance to PM, LR and YR in field trials, while a detached leaf assay with primary seedling leaves indicated a race non-specific nature of the resistance to PM. To determine the time point when the resistance of *Pm38* and *Pm39* becomes active, colony numbers and sporulation rate were measured after inoculating leaf segments taken from plants spanning the whole range of growth stages from 1st leaves of 12-day old seedlings to flag leaves of adult plants. A significant reduction in colony numbers associated with *Pm38* and *Pm39* was only detected in flag leaves around the time for the onset of leaf tip necrosis, while some reduction in the sporulation rate was observed on penultimate leaves. Both genes were associated with partial hypersensitive cell death at the seedling stage, which was observed only for a low proportion of the penetration attempts, and this did not result in any measurable effect on resistance components.

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**P271**

**The floral integrator *WFT* in wheat: expression profiles of three homoeologous genes**

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Flowering is a very important event that is crucial for reproductive success in higher plants. There are four flowering pathways in Arabidopsis such as photoperiodic pathway, vernalization pathway, autonomous pathway and gibberellin pathway. *FLOWERING LOCUS T (FT)* known as florigen plays a central role in flowering of Arabidopsis, and the *FT* homologue in many species have been isolated and analysed until now. In wheat (*Triticum aestivum* L.), the following three components of flowering exist: photoperiod sensitivity, vernalization requirement and earliness *per se*. It has reported that flowering induction under long day condition is occurred by high level of *WFT (Wheat FT)* expression. Wheat is a hexaploid species with AABBDD genome. Wheat genome, therefore, contains three homoeologous genes derived from A, B and D genomes. And then, the expression of *WFT* may include that of three, two or one homoeologous genes. To investigate the expression levels of each *WFT-A*, *WFT-B*, and *WFT-D*, we carried out expression analysis using cv. Chinese Spring (CS) grown under long day or short day conditions. The expression of *WFT* was started at 6 weeks or 8 weeks after sowing under long day or short day conditions, respectively. Interestingly, both under long day and short day conditions, the expression level of *WFT-B* was highest of three homoeologous genes. On the other hand, it has reported that chromosome substitution line CS (Hope7B) showed the early flowering (*Vrn-B3*) in compared with CS. In addition, the flowering time in Hope is later than CS. Now, we are analysing the further expression profiles of *WFT-A*, *B*, and *D* in CS, Hope, and CS (Hope7B), to investigate why the flowering time differ among these three lines.

**P272**

**Coordinated regulation of enzymes involved in secondary cell wall biosynthesis by a homeodomain transcription factor**

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Homeodomain-leucine zipper transcription factors (HDZip TFs) are known to be modulators of morphogenesis in response to environmental stimuli and plant development programs. We have isolated several HDZip TFs from liquid endosperm of wheat at 3-6 days after pollination (DAP) using the yeast one-hybrid system and the synthetic *cis*-element -CAAT(G/C)ATTG- as bait (Lopato *et al.*, 2006). Constitutive over-expression of one of them, TaHDZipII-1, in transgenic barley leads to plants with greatly reduced growth rates, lower numbers of shoots and smaller spikes compared to control plants. Scanning electron microscopy shows that the epidermal cells in the stem of transgenic plants are about 2-3 fold longer than in control barley plants. Microscopic examination of stem sections revealed collapse of cell walls in vascular bundle sheath extension cells, similar to the irregular xylem phenotypes (*irx*) previously described for mutants in genes encoding enzymes involved in secondary cell wall and particularly lignin biosynthesis. The transgenic barley plants have reduced stem strength compared to control plants, although stems of transgenic plants are thicker. Staining with phloroglucinol revealed no lignin in stems, and low levels in vascular tissue in grain and bracts (lemma and palea) in the transgenic plants showing the strongest phenotype. Q-PCR comparisons of gene expression in control and transgenic plants revealed strong and coordinated down-regulation of several genes encoding enzymes specific to secondary cell wall biosynthesis. These included coumarate ligase and laccase, enzymes involved in lignin biosynthesis pathway, as well as several

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cellulose synthases: HvCesA4, HvCesA8, and HvCesA7. All the data suggest that TaHDZipII-1 may function as a negative regulator of secondary cell wall biosynthesis. The transgenic analysis of this gene has been undertaken in barley but transgenic wheat plants are now also being produced and are expected to show a similar phenotype.

**P273**

**Gene expression profiles of hybrid necrosis in synthetic hexaploid wheat**

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Some abnormal growth phenotypes including hybrid necrosis are often observed in F<sub>1</sub> hybrids (genome constitution, ABD, 2n=21) and synthetic hexaploid wheat lines (AABBDD, 2n=42) artificially produced between tetraploid wheat (AABB, 2n=28) and *Ae. tauschii* (DD, 2n=14). The hybrid necrosis phenotypes are generally divided into type I and type II necrosis. In the hybrids plants showing type I necrosis, cell death occurs gradually from the older tissues. On the other hands, the hybrid plants showing the type II necrosis normally grow until exposure to low temperature. Little information about causal genes of hybrid necrosis has been reported, and the biochemical and molecular mechanisms of the hybrid necrosis are still largely unknown. Our study showed that *Ae. tauschii* accessions inducing hybrid necrosis were phylogenetically and geographically biased, indicating that the two types of hybrid necrosis might be independently induced by unknown genetic factors according to the Dobzhansky-Muller model. To compare comprehensively gene expression profiles among the synthetic hexaploid wheat lines showing the normal growth (WT) and type I necrosis phenotypes, cDNA-AFLP analysis was performed using mRNAs from the four synthetic hexaploid wheat lines between tetraploid wheat cultivar Langdon (Ldn) and *Ae. tauschii* accessions, and their parental lines. Totally 777 AFLP fragments were observed using the 55 selective primer sets. Ldn- and *Ae. tauschii*-derived fragments and newly appeared in the synthetic wheat were observed and compared between the WT and type I necrosis lines. However, no significant differences were found in the number of the Ldn- and *Ae. tauschii*-derived fragments the newly appeared between the WT and type I necrosis lines. Until now, few genes specifically expressed in the WT and type I necrosis lines could be identified, which indicated that limited changes of the gene expression patterns might induce the phenotypic difference between the WT and type I necrosis lines.

**P274**

**Genetic mapping of a new flowering time gene on wheat chromosome 3B**

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A new flowering time gene, possibly an *eps* effect, which had been previously reported by the authors from studies of substitution lines of chromosome 3B of the Czech alternative wheat variety Česká Přesívka (CP 3B), was genetically mapped using two mapping populations. Recombinant substitution lines were derived from SSR validated substitution lines of Sandra (CP 3B) and Zlatka (CP 3B). Comprehensive genetic maps of chromosome 3B were developed using SSR markers. The flowering times of individual lines of the mapping populations was measured in five experiments grown under different experimental regimes, two for the Sandra (Sandra 3B/CP 3B) population and three for the Zlatka (Zlatka 3B/CP 3B) population. QTL analysis was carried out on each experiment and a single flowering time QTL was mapped in both populations into a position near the centromere on the long

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arm of 3B, in the region near the marker locus *barc164*, having an additive difference of 1-6 days, depending on environment and genetic background. These genetic maps of 3B were compared with other 3B maps developed by the authors based on recombinant doubled haploid populations of UK winter wheats, Spark x Rialto and Charger x Badger. This comparative analysis revealed a common flowering time QTL with a suggested symbol *QFt.CRI-3B.1*, segregating in diverse European wheat germplasm. The work was supported by the research plan MZe ČR 0002700602.

## **P275**

### **Macroarray for studying chloroplast gene expression profiles associated with the initial development of wheat**

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Chloroplasts, as photosynthetic carbon assimilating organelle, play an essential role in plants throughout their development. We have previously devised mitochondrial macroarray using a whole set of rice and wheat mitochondrial genes to study their expression associated with biogenesis and initial development of this energy producing organelle in wheat. We now report the development of chloroplast macroarray that can potentially provide us with wide-applicability in studying chloroplast gene expression in wheat and other cereal crops. Chloroplast macroarray was developed using 60 wheat chloroplast genes (excluding tRNA and small size genes). In addition, 5 nuclear encoded chloroplast-targeted genes and 2 control nuclear genes were spotted on nylon membrane. Primer sets were designed based on the published wheat chloroplast genome sequence (Ogihara et al., 2000). Changes in gene expression profiles were monitored using RNA isolated from seedlings at four different stages of development, i.e. germination through 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> leaf stages. Total RNA was extracted with Sepasol-RNAI and cDNA was synthesized using random hexamers and ReverTra Ace. cDNA labelling and signal detection was according to protocols of ECL Direct Nucleic Acid Labelling and Detection Systems (GE Healthcare). The data were evaluated with ImageJ software, and the transcript amounts were normalised against actin transcript. The macroarray system revealed remarkable differences and dynamic changes in the level of individual transcripts during the initial stages of wheat development. The levels of *psbA*, *psbO*, *rpoB* transcripts increased until the 3<sup>rd</sup> leaf stage, *rbcL* and *rbcS* transcript levels remained unchanged, while those of *psaA*, *psaB*, *psaC* showed considerable fluctuations with an increase at the 1<sup>st</sup> leaf stage. Hybridisation was also successful with cDNAs of barley and rice, suggesting the versatility of this macroarray system for studies of chloroplast biogenesis in Gramineae.

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**P276**

**Creation of a multiple-use recombinant inbred line population for the development of molecular markers in soft white winter wheat**

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To develop molecular markers for soft white winter wheat in the most economical way possible, a community approach was taken to develop a recombinant inbred line (RIL) population for mapping traits in soft white winter wheat and to generate the molecular and phenotypic data necessary to identify useful molecular markers. Parental selection was done to maximize differences for multiple traits of interest instead of targeting one or two specific traits. Two commercially grown cultivars, Coda and Brundage, were selected based on differences in disease resistance, abiotic stress tolerance, agronomic traits and end-use quality to produce the population CB-2. To improve the potential for identification of useful molecular markers 270 F<sub>6,7</sub> derived lines were developed for phenotypic screening. Initial molecular analysis of the two parents identified 220 polymorphic SSR markers and 180 DArT markers. To economically produce the molecular and phenotypic data, different wheat breeding programs have taken the lead on phenotypic screening for specific traits and are pooling mapping data to minimize costs to any specific program and to rapidly map the population. To date, transgressive segregation has been observed in the population for resistance to *Puccinia striiformis*, tolerance to *Cephalosporium gramineum*, and height. The population has been used to identify new SSR markers for the *Pch1* resistance gene and the *C* gene related to spike compaction. Complete molecular mapping of the population is anticipated by the end of 2008. To maximize use of the population by other programs, all molecular marker data and agronomic data associated with the CB-2 RIL population will be publicly available.