

**P219**

**Investigation of Zinc uptake efficiency in six variety of Iranian bread wheat**

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Keeping in view the significant role of zinc in qualitative and quantitative characteristics of Iranian bread wheat and especially in wheat flour fortification which in turn affects human health, it is quiet necessary to use varieties with higher Zinc uptake efficiency. The present investigation was conducted during 2006-7 to meet the same objective at a field with moderate texture and insufficient nutrients especially Zinc. The experiment was laid out in a randomized complete block design with tree replication. Eighteen combinations of two factors including tree level of Zinc application i.e. 0, 40 and 80 kg ha<sup>-1</sup> Zinc sulphate and 6 variety of wheat were investigated. Various physiological indices were studied. Result revealed that application of 40 kg per ha Zinc sulphate could significantly improve many of these indices including: plant height, test weight, grain and protein yield as well as harvest index. Triticale had significantly higher Zinc uptake efficiency (97%), which durum wheat showed the least (66%). Four bread wheat varieties stand among them. It may be concluded that wheat and rye hybridization could significantly raise Zinc uptake.

**P220**

**Molecular characterization of *Cyclophilin B* genes and promoter sequences in wheat and rice**

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Cyclophilin B belongs to the PPIase (peptidyl prolyl isomerase) enzyme group that catalyses the rate-limiting step of *cis-trans* isomerization of X-Pro peptide bonds in nascent proteins. Cyclophilins are up-regulated during endosperm development and suggested to be involved in storage protein folding and deposition, thus potentially influencing grain quality. However, little genetic information exists on this 'foldase' enzyme, and on the flanking regulatory sequences which might affect the timing, tissue specificity and transcription rates. The *CypB* genes from common wheat (*Triticum aestivum* L., AABBDD) were cloned and characterised and their genomes identified by comparison with genes isolated from the progenitors of wheat. The full-length gene *TaCypB-B* is >2.6 kb long and contains seven exons and six introns. The putative CypB protein (213 amino acids) contains all PPIase signature sequences including the active site and cyclosporin-binding residues, but an atypical ER localisation signal. It shares 83.2% identity with the putative CypB protein (220 amino acids) of rice encoded by the gene identified from the TIGR rice genome database. The 5' and/or 3'-flanking regions of the wheat *CypB* genes were obtained in the present study by inverse PCR (IPCR) from *SacI*-digested and ligated genomic DNA of *Ae. tauschii* (DD) and *T. urartu* (AA). Rice *CypB* 5'-flanking sequence was identified from the rice TIGR genomic database. Alignment of putative *CypB* promoter sequences of *Ae. tauschii* and *T. urartu* showed 75.9% identity over a 500bp region upstream of the translation start codon and 88.5% identity over the -370bp upstream region. Analysis of promoter sequences in *T. urartu*, *Ae. tauschii* and rice showed they had no TATA-box, but had the GC-rich sequences for transcription start site (TSS). In addition, a number of putative regulatory elements were identified, including endosperm motifs for endosperm specific expression, DOF cores

and E-boxes for tissue specific expression, AT-rich motifs for enhancing seed specific expression and CT-leader boxes influencing the gene expression quantitatively. The core promoter sufficient for the transcriptional activation of *CypB* was also identified. The results provide essential data for studies on gene expression, genetic mapping and testing for association with quantitative trait loci related to protein quality.

### P221

#### Genetic and ecophysiological analysis of grain protein deviation in winter wheat

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The negative relationship between grain yield and grain protein concentration is a well-known phenomenon in wheat and to a larger extent in many crops. Breeders have tried for a long time to outcome this relation in order to improve these two traits simultaneously but few significant results have been obtained. Recently, it has been proposed to select lines that significantly deviate from the relation. This selection criterion has been called Grain Protein Deviation (GPD). The aim of our study is to identify the ecophysiological and genetic determinism of GPD. To assess the ecophysiological determinism of the negative relationship between grain yield and grain protein concentration, we propose to characterize the nature and the intensity of the limiting factors observed in the different growing environments. We then propose to look for physiological traits influencing GPD and to realize a QTL detection for GPD and these traits in different mapping populations grown in various years, locations and nitrogen supplies. Preliminary results showed a high environmental variability for the slope and correlation coefficients of the relation. Nevertheless, it was possible to identify lines that deviated positively or negatively from the relation regardless of the growing environment. Several QTL for GPD, grain yield and grain protein concentration have been detected in the different populations studied. QTL detection for these three traits in different environments allowed the identification of pleiotropic QTL and environment by QTL interactions. Moreover, despite the strong correlation between GPD and grain protein concentration, it was possible to detect QTL that affected only GPD. This study will offer new insights into the relationship between grain yield and grain protein concentration and provide new traits for breeders who want to outcome this negative relationship.

### P222

#### Flour yield and water absorption in wheat – a pedigree mapping approach

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Flour yield and flour water absorption are two of the most important quality traits for milling wheats. A number of studies have tried to locate QTL for these traits, using doubled haploid mapping populations. Results from these studies suggest that these traits are determined by genes in different parts of the genome in different populations, and that large genotype by environment interactions may be occurring. A pedigree mapping approach may be more amenable for genetic analysis of these traits. This approach uses knowledge accumulated for related breeding lines from an improvement program, and incorporates data from a number of years and trial sites. Using this method, we were able to identify more robust QTL for flour yield and water absorption within the breeding populations. We were also able to identify which lines within the pedigree were carrying the markers for high

quality donor alleles, identical by descent, and are thus more likely to carry the desirable genes. The identified QTL were compared with those found in other studies, as further validation. The relationship between flour yield and flour water absorption, and between milling yield and seed size was also investigated. The often reported negative relationship between flour yield and flour water absorption has made it difficult to breed for both traits simultaneously. However studies have shown that lines exist which combine both high milling yield and high water absorption, but these lines are rare. Because phenotypic screening for quality traits generally does not commence until late in the selection process, there is a high probability of lines with high flour yield and high flour water absorption being discarded. If markers could be applied early in the selection process to enrich breeding populations for these desirable genes, step-wise progress could be made for important traits.

### P223

#### A comparison of methods for assessing polyphenol oxidase status of wheat

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Polyphenol oxidases are involved in the time-dependent darkening and discolouration of Asian noodles and other end products of wheat. It is desirable to select low PPO wheats. In this study we compared the catechol and the L-tyrosine methods for assessing PPO activity in seed, using visual and single and dual optical density readings. We compared these results with marker profiles for the QTL on chromosome 2AL previously identified by Raman et al as explaining a large percentage of variation for this trait. We found that catechol gave a lower absorbance reading than tyrosine, and dual readings gave a lower reading than single readings. Catechol readings were generally more consistent than tyrosine between replicates. Correlations were generally high between single and dual reads. Correlation between the catechol and tyrosine methods was high for visual scoring, but poorer for optical density readings. In general visual assessment was better correlated with optical densities for the tyrosine method than with the catechol method. The ranking of our 3 standards differed between tyrosine and catechol methods. There was some suggestion that there may be some environmental influence on PPO levels expressed in the seed. There was a near perfect correlation between visual scores after catechol treatment and marker scores, and a high correlation after tyrosine treatment, with only a few discrepancies in middle rated lines. Our conclusion is that though each of these methods can be useful for selecting lines for low in PPO in selection programs, where molecular markers are already being applied for selection, the application of markers is likely to be the most cost effective strategy for enriching a population for low PPO.

### P224

#### The protein disulfide isomerase (PDI) gene family in wheat

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The PDI family includes several genes controlling diversified metabolic functions, the most important consists in disulfide bond formation and isomerization during protein folding, which is accomplished by two thioredoxin like active sites. In plants the PDI family includes eight phylogenetic classes. In wheat the characterization of the three homoeologous gene sequences encoding typical PDI (*TaPDIL1-1*) and of their promoter sequences has been reported previously. The aim of the present research is the study of the complexity and diversity of the PDI gene family in wheat. A cross search of rice PDI-like sequences in EST databases of wheat found eight additional homologous sequences, whose full length cDNAs were cloned. Phylogenetic analysis assigned the nine PDI and PDI-like sequences of wheat to the eight plants phylogenetic groups. Conserved motives were searched by

comparison with sequences in different protein databases. The deduced amino acid sequences of the eight cloned genes revealed a high level of structural similarity among the proteins encoded by genes belonging to the same phylogenetic group. The comparison of the genomic organisation of three wheat PDI-like genes (*TaPDIL2-1*, *TaPDIL4-1* e *TaPDIL5-1*) with their orthologous of rice and *Arabidopsis* showed a high level of conservation of their structural features (exon/intron structure, exon length and position of the active sites) among members of the same phylogenetic group. Most likely such structural conservation reflects the key functional role of their products. Further analyses of the novel PDI-like cDNA sequences will include: 1) evaluation of their copy number per genome and chromosomal location by Southern analysis; 2) quantitative expression analysis by real-time PCR in different plant tissues, including developing caryopses; 3) quantitative expression analysis of seedlings grown at low (5 °C) and high (35 °C) temperatures for 24 and 48 h.

### P225

#### Association mapping of semolina yield in diverse durum wheat germplasm

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Semolina yield is an important end-use quality attribute of durum wheat (*Triticum turgidum* L. var *durum*), and high semolina yield is preferred by millers and processors. Semolina yield is difficult to assess in breeding programs because of slow measurement throughput and the large grain sample requirement for accurate measurement. The trait is therefore a good candidate for marker-assisted selection. As a first step toward this goal, we performed genome-wide association mapping (AM) for semolina yield to identify genomic regions for more detailed investigation. Eighty-two genetically diverse durum genotypes were scored for 245 microsatellite markers and assessed for semolina yield at two locations over two years. There was good genetic variation for semolina yield, ranging from approximately 61.5 to 70.5%. Marker associations for semolina yield were identified on chromosomes 2A, 3A, 4A, 4B, 5A, 6B, 7A, and 7B, strongly indicating quantitative inheritance. Flour yield QTL have also been reported on chromosomes 3A, 4B, 5A, 6B and 7A of hexaploid wheat, adding support to these results. Doubled haploid lines from an inter-cross of a high and a low semolina yield line have been generated and will be genotyped and screened for semolina yield to further investigate the genome regions identified in the AM study.

### P226

#### A molecular toolbox for Xanthophyll genes in wheat

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The biological accumulation of xanthophyll compounds responsible for yellow colouration of the wheat endosperm is of considerable commercial interest due to varying flour colour requirements for

specific end use products. The xanthophyll biochemical pathway is comprised of around nine major gene groups and associated enzymes involved in the synthesis of various xanthophyll or carotenoid components. The current study seeks to establish a molecular toolbox for exploring the chromosomal location and expression of xanthophyll related genes. Identification of target genes has involved online database searches for gene ontologies and similarity searching with rice or arabidopsis to retrieve wheat sequences. To include the possibility of multiple genes within each group, primer design is based upon the alignment of sequences from related grain species and validated by phylogenetic assessment. Genes were assigned to chromosomal regions using nullisomic-tetrasomic and deletion line analysis and were used to align with QTL for flour b\* and xanthophyll from a range of mapping populations. A gene encoding a putative enzyme for geranylgeranyl transferase 1  $\alpha$  subunit was mapped to the short arm of chromosome 3B and 3D. This gene mapped to a similar region containing a QTL for flour b\* and xanthophyll content identified in a Ajana/WAWHT2074 DH mapping population. The chromosomal location and biological nature of the genes will assist in the identification of functionally associated markers and allelic variation for xanthophyll content and flour colour in Australian wheat germplasm.

### P227

#### The effects on grain quality traits of a grain serpin protein and the VPM 1 segment in southern Australian wheat breeding

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Identification and evaluation of alleles of genes impacting on wheat quality enables breeders to improve their germplasm by selection toward specific allele combinations. Using a large data set obtained from southern Australian wheat breeding programs, and including a relationship matrix in the analysis to minimise biases, we evaluated the effects of a defence grain protein, a serpin located on chromosome 5B, and the VPM1 alien segment on the grain quality parameters Rmax, dough extensibility, dough development time, flour water absorption and milling yield. The data spanned the period from 1983 to 2006 and included data from 899 lines in 545 environments. The serpin null allele significantly reduced milling yield by approximately 0.4g of flour per 100g of grain milled across different germplasm sources and flour protein levels. In Australian germplasm, the origin of this allele was traced to a 19<sup>th</sup> century introduction from India by William Farrer. However, other sources, of significance in international breeding programs, were also identified. Our analysis found no detrimental effects of the VPM1 alien segment on the quality traits we measured.

### P228

#### Revealing the genetic relationship between dough rheology and loaf volume using QTL analysis of mixograph traits in wheat

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Loaf volume is a direct parameter of wheat bread making quality and mixograph is routinely used to test and evaluate the loaf volume in determining the quality & functionality of wheat. Identification of genetic markers linked with traits was attempted using an RIL population developed from a cross between two Indian wheat varieties “HI977” and “HD2329”. The phenotypic data of these traits were collected from six environments including three different agro climatic zones for two consecutive years, separately for loaf volume and mixograph traits. Loaf volume was measured using straight dough method and mixograph experiments with 10g grain flour using standard mixograph instrument. The composite interval mapping revealed 169 QTLs for 9 mixograph traits such as envelope peak integral, mixing peak time, midline right integral, midline curve tail integral, midline curve tail value, midline curve tail width and weakening slope. The mixograph QTLs clustered with loaf volume QTLs on chromosomes 2A, 5D, 6B, 6D revealing the genetic relationship of mixograph traits with loaf volume.

### **P308**

**Composite interval mapping and stability analysis for bread making quality and yield traits in wheat (*Triticum aestivum* L.)**

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Sedimentation volume and Grain protein content have remained as indirect parameters to measure bread making quality, while thousand grain weight and test weight as indicators of wheat marketability. Identification of genetic markers linked with these traits was attempted using an RIL population developed from a cross between two Indian wheat varieties “HI 977” and “HD2329”. The phenotypic data of these traits was collected from six environments including three different agro climatic zones for two consecutive years. The composite interval mapping revealed 68 QTL controlling sedimentation volume, grain protein content, thousand grain weight and test weight with a total of 9 QTL clusters on 7 chromosomes, of which, two clusters were on chromosomes 1B and 5D. Stability analysis was attempted using additive main effect and multiplicative interaction model was used to select stable RILs for various traits. It revealed significant contribution of genotype x environment variance due to all traits. These stable RILs were clustered based on alleles, flanking the QTLs controlling these traits and an attempt was made to observe a pattern of the QTLs.

### **P229**

**Cytokinins contents and dry matter accumulation at different position and types of grains within a spike of wheat (*Triticum aestivum* L.)**

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## Coping with wheat in a changing environment – wheat quality: poster abstracts

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Dry matter accumulation and cytokinin (zeatin and zeatin riboside) level of grains in various positions within the wheat (*Triticum aestivum* L. var. PBW-343) spike and spikelet were investigated during the grain filling period. Main shoot ears were partitioning into proximal, middle and distal regions and further into bold (basal) and Small (apical) grains. Ten labeled spikes were sampled every 4 days from 3 days after anthesis (DAA) to 23 DAA, and every 7 days from 23 DAA to maturity. The methods for extraction and purification of zeatin and zeatin riboside were modified from those described by Bollmark et al. (1988) and He (1993). Results have shown that the cytokinin level increased rapidly from about 7 until 15 days after anthesis and then decreased depend upon the position of grains in spike and spikelet. The differences in cytokinin levels, both among spikelets in different regions of the spike (i.e. proximal, middle and distal) and also among grains within a spikelet (i.e. basal and apical), were positively correlated with the differences in dry matter accumulation. Higher zeatin and zeatin riboside contents in the grains at the early grain filling stage, may promote the division of endosperm cells, thus constitute a powerful sinks, and enhance assimilate migration and its accumulation in the developing grains. Therefore, it might be possible to improve grain filling by increasing cytokinin levels in grains, especially at the early filling stage either through breeding or crop management. The results suggest that cytokinins in the grains during the early phase of grain development play an important role in regulating grain filling pattern and consequently influence grain filling percentage.

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2-He Z (1993) Guidance to experiment on chemical control in crop plants. Beijing: Beijing Agriculture University Publisher, pp 60-68

**P230**

**Relationship between yellow alkaline noodle quality and flour properties in Japanese hard wheat**

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Most of Japanese wheat variety is soft wheat which is mainly used for Japanese noodle. Recently, however, we have started breeding hard wheat varieties for yellow alkaline noodles (YAN) or bread. In this study, the effects of protein content, amylose content, and dough properties on YAN quality were investigated in Japanese hard wheat. Protein content was highly correlated with noodle colour, but the colour of noodles made from white seed varieties was superior to that of noodles made from red seed varieties. Thus the seed colour is one of the important factors for YAN quality. The texture of noodles made from low amylose type varieties was very smooth. This texture is useful for new noodle products. The hardness and elasticity of noodle after immersion in soup for 8 minutes, which are important determinants of YAN quality, were highly correlated with the protein content and dough properties, as evaluated using a farinograph. Compared with Australian Prime Hard, which is suitable for YAN, Japanese hard wheat with high molecular weight (HMW) glutenin subunits of Japanese noodle wheat varieties was not suitable for YAN. These results indicated that YAN quality in case of Japanese hard wheat can be improved by genetically introducing of HMW glutenin subunits which effectively strengthened the dough property (e.g. *Glu-D1d*, 5+10 subunit).

**P231**

**Protein polymer accumulation during grain development and relations to quality:  
Influences of cultivar and environment**

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Bread baking quality of wheat is influenced by protein concentration and composition, and amount and size distribution of polymeric protein (ASPP). Reasons for variation in ASPP in mature wheat are not fully understood and can be searched for during grain development. The aims of our investigations were to evaluate cultivar and environment influences on protein polymer composition in mature wheat, to investigate general built-up patterns of polymeric proteins, and to explain alterations by cultivar and environment. Protein concentration, composition and polymerisation, and bread-making quality have been determined in spring and winter wheat of different cultivars grown in climate chambers, green-houses and on field during grain-filling and at maturation. The results show that;

- Gluten strength is influenced by protein concentration, specific protein composition and ASPP
- Protein concentration and ASPP is influenced by cultivar and environment, while specific protein composition is determined by cultivar
- Rate of nitrogen is influencing protein concentration, while timing of nitrogen application influences gluten strength and ASPP
- Temperature together with nitrogen availability and cultivar determined development times are the main factors influencing ASPP
- Grain moisture are an important parameter for protein polymerisation in the developing wheat grain

Thus, the general protein accumulation pattern in wheat is a pre-determined event, although ASPP is influenced by cultivar, temperature and nitrogen availability during grain development leading to differences in the mature grain.

### P232

#### Characterisation of puroindoline genes in wild tetraploid and hexaploid wheats (*Triticum araraticum*, *T. timopheevii* and *T. zhukovskyi*)

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Puroindolines are small basic, cysteine-rich proteins found in the caryopses of many taxa of the Triticeae tribe. They belong to a protein super-family that includes alpha-amylase/trypsin inhibitors, nonspecific lipid binding proteins and a mixture of puroindoline-like polypeptides (Grain Softness Proteins, GSPs). In recent years, puroindolines have gained a considerable interest among wheat geneticists and breeders, offering new perspectives in the genetic improvement of cereals, spanning from end-use applications to disease resistance. Remarkably, tetraploid wheats (AABB) have repeatedly shown to be devoid of puroindolines, whereas other major polyploid lineages of *Triticeae* have been little inspected and with contrasting results. In this paper we report the discovery and characterisation of puroindoline Pin A and Pin B genes in some wild tetraploid and hexaploid wheats [two accessions of *Triticum araraticum* (AAGG), one accession of *T. timopheevii* (AAGG), and one accession of *T. zhukovskyi* (AAAAGG)], where the absence of the *Ha* locus had been previously postulated due to unsuccessful isolation of the gene sequences. The four Pin A sequences displayed an overall 99.89% nucleotide identity: the three tetraploid accessions were 100% identical, whereas *T. zhukovskyi* showed two nucleotide substitutions. Identity with bread wheat (Chinese Spring) ranged between 98.2 and 98.7%. The four Pin B sequences displayed an overall 99.61% nucleotide identity, and identity with Chinese Spring ranged between 93.7 and 94.2%; the deduced main features of the protein secondary structures were all maintained. Exception is one accession of *T. araraticum* which showed the lack of a cysteine in Pin B, for which we can assume disruption of the C-backbone with the consequent loss of functionality.

### P233

#### Use of mutagenesis to induce novel allelic variation for genes involved in starch biosynthesis

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Given the important role starch plays in food and non food uses several efforts are being made to manipulate its composition through modification of the amylose/amylopectin ratio in different crops in general and in wheat in particular. Approaches used to achieve this goal are being pursued through the manipulation of the genes involved in the starch biosynthetic pathway using natural or induced mutations and transgenic methods. The use of mutagenesis to produce novel allelic variation represents a powerful tool to increase genetic diversity and this approach seems particularly suitable for starch synthase genes for which limited variation exist. In this work M<sub>4</sub> EMS treated seeds of the bread wheat cv Cadenza have been used and mutations generated by the treatment have been identified by combining SDS-PAGE analysis of granule bound starch proteins and through a TILLING approach at the gene level. In particular we have focused on two groups of synthase genes, those encoding the starch synthase II (*Sgp-1*) and those corresponding to the waxy proteins. *Sgp-1* proteins are involved in amylopectin biosynthesis, whereas waxy proteins are unique isoforms implicated in amylose biosynthesis. In bread wheat three different *Sgp-1* proteins are present, which

are encoded by three genes located on chromosome arms 7AS, 7BS and 7DS; similarly three waxy proteins are encoded by three genes located on chromosome arms 7AS, 4AL and 7DS. SDS-PAGE analysis of granule bound proteins has allowed the identification of *null* genotypes at all the analyzed loci. Molecular characterization of induced mutants has been performed using gene-genome specific primer pairs for *Sgp-1* and *Wx* genes. New *Sgp-1* alleles have been identified at the different homoeoloci by TILLING. In particular single nucleotide substitutions, introducing a premature stop codon or amino acid modifications, have been identified.

### P234

#### PCR-based markers for starch synthase genes in wheat

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Starch composition in wheat endosperm has an important effect on starch quality. There are about 25% amylose and 75% amylopectin in bread wheat, but in some cultivars, the enzymes which catalyze synthesis of amylose and amylopectin may be deleted. These deletion mutants can be used to breed new cultivars which contain very high or very low amylose. For example, Waxy wheat lacks all of the waxy proteins (granule bound starch synthase), and its amylose content is less than 1%. Starch synthase is a major enzyme in amylopectin synthesis. There are three homoeologous proteins: SS -A1, SS -B1, and SS -D1 in bread wheat. In this study, 5 pairs of primers were designed for SS alleles based on the *ss* gene sequences (genebank accession: AB201445, AB201446 and AB201447), in which 2 pairs were specific for *ss -a1*, 2 pairs were specific for *ss -b1* and 1 pair was specific for *ss -d1*. Chinese spring and its 6 nullisomic-tetrasomic lines were used to validate the accuracy of the primers, which showed that these primers could be used to distinguish alleles of SS and accelerate starch quality breeding by marker assisted selection.

### P235

#### Tissue specific promoters from rice and wheat for modifying grain characteristics

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Grain-specific promoters are essential tools for the improvement of grain quality using transgenic approaches, or for functional genomics studies of grain development. We identified and cloned several promoters from rice and wheat, which are active either in different grain tissues or in early grain and flowers shortly before fertilization. Transcriptional GUS fusion constructs were transformed either into rice and barley using *Agrobacterium tumefaciens*-mediated transformation, or in wheat using microprojectile bombardment. Three of promoters, designated *OsPR602*, *OsPR9a*, and *TdPR60*, were activated specifically in endosperm transfer cells and adjacent starchy endosperm in wheat and barley starting from 9 days after pollination. These promoters offer the potential to improve of grain quality by modifying the quality and quantity of nutrient transfer to the grain. They may also be of value in enhancing the efficacy of this tissue as a barrier to pathogen movement from maternal tissue to the developing endosperm. The *TdPR61* promoter activity was localised to the region surrounding the embryo as early as 6 DAP and in embryo at later stages of grain development. It can be used to engineer sterility through the early grain abortion. *OsPRPI* and *TdPRPI* promoters are active in female gametophyte before fertilisation; the maximum activity was detected in aleurone and adjacent to aleurone cell layers at 5 to 7 DAP and decreased at 15 DAP. These promoters will be useful for improvement of the disease resistance at early stages of grain development and in minimising grain abortion under stress. Another promoter, *TdPRGL7* is also activated before fertilization. The GUS

expression was detected in endosperm and the promoter was active until the 40 DAP. The promoters have been cloned in the pMDC32 vector and obtained vector derivatives can be used for the transformation of rice, barley and wheat to target gene expression to different grain tissues.

### **P236**

#### **Determination of relationship between HMW glutenin subunits and bread making quality in bread wheat**

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High molecular weight (HMW) subunits of wheat glutenin are generally considered to play a key role in gluten formation and structure, and to be closely related to wheat quality. The endosperm storage proteins of 80 synthetic lines and wheat cultivars (*Triticum aestivum*) were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis to determine their high-molecular-weight (HMW) glutenin subunit composition and relationship with bread-making quality. twenty six high-molecular-weight (HMW) glutenin subunit composition including fifteen different alleles were found which 2\* allele of Glu-A1, 7+8 allele of Glu-B1 loci and 5+10 of Glu-D1 were of higher frequency. 2\*\*\*+12 subunit, first reported from Pakistan, was observed in 7 line. SDS sedimentation test was performed to studying the effects of HMW Glutenin subunits on bread-making quality. The results of ANOVA showed that 2\*, 5+10 and 17+18 alleles of Glu-A1, Glu-D1 and Glu-B1, had the most positive effects and Null allele(43.75%) had negative effects on SDS sedimentation trait. Stepwise regression analysis revealed that the subunits of 5+10, 17+18 and 7+8 can justify 31/4% of variation in SDS sediment.

Key words: HMW-GS, bread wheat, SDS sediment test, bread-making quality.

### **P237**

#### **Varietal differences in protein polymer built-up of wheat at different temperature and nitrogen regimes during grain filling**

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The amount and size distribution of polymeric proteins, influenced by cultivar, temperature and nitrogen timing, is an important factor in determining gluten strength in wheat. In mature wheat grains, the distribution of the monomeric and polymeric proteins as well as their solubility play a critical role in governing wheat flour properties and uses. Four varieties of wheat (two early and two late), differing in high-molecular-weight glutenin subunit composition (2+12 versus 5+10) were grown at four nitrogen regimes and two temperature regimes in order to determine the manner in which differences in mature protein composition were the result of differences in accumulation of proteins during grain filling. Combination of temperature and nitrogen regimes leads to changes in amount and size distribution of polymeric proteins in mature grains of wheat. A relationship is present between polymerisation of proteins during grain maturation and amount and size distribution of polymeric proteins in the mature grains.

**P238**

**Control of grain constituents involved in colour and colour stability in Asian noodles**

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Colour and colour stability are important quality attributes of Asian noodles made from bread wheat (*Triticum aestivum* L.) flour that strongly influence consumer acceptance and therefore market share. Whilst colour and colour stability can be determined relatively simply for any batch of flour, these traits each represent a complex of independent components controlled by genotype and the environment in which the grain was produced. In addition, the relative importance of these components varies depending on the style of noodle; the 2 main types being white salted noodles (WSN) and yellow alkaline noodles (YAN). Logically, efforts to improve the noodle quality of wheat cultivars should focus on the individual components rather than colour *per se*. Initial colour can be separated into brightness (or whiteness) and yellowness or creaminess. Initial brightness is determined primarily by the granularity of the flour and protein content. Yellowness is controlled by lutein and lutein ester content (WSN and YAN) and by apigenin-C-diglycoside content (YAN). Colour stability involves loss of brightness or yellowness or both with time. Lipoxygenase activity rapidly degrades lutein in WSN but is inactive in YAN. Loss of brightness, darkening, involves the dark pigments generated by polyphenol oxidase (PPO) and non-PPO reactions. In addition, yellowness or creaminess is progressively masked by these dark pigments. Simple phenotyping methods have been developed and significant genetic variation, QTL and linked markers identified for most of the components contributing to colour and colour stability.

**P239**

**Detection of QTLs for heat tolerance in wheat measured by grain filling duration**

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Heat stress is a major environmental stress limiting wheat productivity in most cereal growing areas of the world. In order to map and characterize quantitative trait loci controlling heat tolerance, 144 recombinant inbred lines derived from the cross of Kauz and MTRWA116 were evaluated by normal and late sowing in a simple lattice design in a very hot area. Grain filling duration was used as a measure of heat tolerance. One hundred and sixty six SSR and 3 AFLP markers were used to construct a linkage map containing 18 linkage groups and covering 16 chromosomes of wheat. With the method of composite interval mapping one major QTL was detected for heat tolerance, measured by grain filling duration, on chromosome 2D. Closely linked to Xgwm484, the QTL appeared in both normal and stressed conditions.

**P240**

**Arabinoxylan content of hard winter and spring wheats of the US Pacific Northwest**

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The development of high quality wheat (*Triticum aestivum* L.) cultivars depends on a thorough understanding of the genetic and environmental influences on the constituents of grain. Arabinoxylans are important albeit quantitatively minor constituents of wheat grain; they can interact with large weight ratios of water and participate in oxidative cross-linking and gel formation. In this study, 26 hard winter and 25 hard spring wheat genotypes from breeding programs in the U.S. Pacific Northwest were analyzed for water-soluble and total arabinoxylan content. Each genotype was grown in three environments. There were significant differences among water-soluble (WS-AX) and total (TO-AX) arabinoxylan contents (G, E, and G\*E model R<sup>2</sup>s 0.64-0.96). WS-AX genotype mean content ranged about 2-fold (0.39 to 0.81% for winter, and 0.48 to 0.92% for spring genotypes). TO-AX genotype mean content ranged from 3.1 to 4.0% for winter and 3.9 to 4.7% for spring genotypes. Type III SS F-ratios for 'genotype' were highly significant (P<0.0001) for both AX fractions of both winter and spring genotypes. 'Hollis' spring wheat had the highest WS-AX content and 'WQL9HALP' spring wheat (a hard NIL to 'Alpowa') had the highest TO-AX content. Repeatability estimates were 0.71 and 0.89 for WS-AX, winter and spring; and 0.30 and 0.62 for TO-AX, winter and spring genotypes, respectively. These preliminary results indicate that there is sufficient repeatable genetic variation to improve wheat cultivars for AX contents.

**P241**

**Bread making quality attributes of Iranian trade cultivars of wheat and their HMW glutenin subunits composition**

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Sixty seven varieties of wheat including mostly hexaploid and durum genotypes grown in Iran were tested for bread making quality attributes and their hectoliter weight, percentage of protein content, grain hardness index, flour water absorption, falling number and SDS-sedimentation volume were determined. High molecular weight (HMW) glutenin subunits composition of these varieties was also determined using SDS-PAGE. Clustering of the investigated varieties was performed using SDS sedimentation volume as a gluten strength index. Two clusters were obtained each included 2/3 sub clusters. First cluster included most of the varieties (43 entries) with SDS sedimentation volume of 42-54 ml. These varieties are considered as moderate quality entries which are suitable for flat bread making. Second cluster which included 3 sub clusters included 24 varieties with SDS sedimentation volume of 55-70 ml, which could be considered as good quality genotypes and favorable for pan bread making or improvement of gluten strength in poor quality cultivars. The HMW glutenin genotype of these varieties is presented. A particular HMW glutenin subunit allele was absorbed in two cultivars originated from crossing of Iranian landraces which was determined as 2/1+10\*, a similar combination reported previously in Afghanistan wheat varieties.

**P242**

**Effect of Gpc-B1 gene in F2 population crossed between Hard Red Spring wheat cultivar and Japanese soft wheat cultivars**

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Japanese wheat cultivar is primarily soft wheat and used for Japanese noodle (Udon). The grain protein content suitable for Japanese noodle is from 10.0% to 11.0%. In Kyushu region of Japan, grain protein content is lower than the suitable one. Therefore, farmers in Kyushu region have to apply more fertilizer than ones in other regions. We evaluate the effect of Gpc-B1 (Grain Protein Content) gene in Japanese soft wheat background to breed new cultivar with high grain protein content even in Kyushu region. Yecora-rojo (HGPC) was the source of Gpc-B1 gene. Chikugo izumi was a Japanese soft wheat cultivar and Saikai 185 was a soft breeding line. The F2 populations from Yecora-rojo (HGPC)/Chikugo izumi and Yecora-rojo (HGPC)/Saikai 185 were grown in experimental field of National Agricultural Research Centre for Kyushu Okinawa Region (Chikugo, Fukuoka, Japan). Grain protein content was determined with Infratec 1241 Grain Analyser. Hardness and thousand kernel weight were determined with Single-Kernel Characterization System (SKCS) 4100. Xuhw84 and Xuhw108 markers showed polymorphisms between Yecora-rojo (HGPC) and the two Japanese soft wheats. These polymorphisms were used to identify the plant with Gpc-B1 gene. In both F2 populations, grain protein content was not correlated with thousand kernel weight ( $r=-0.299$  and  $0.017$ ). Therefore, high grain protein content was not result from low thousand kernel weight. Among plants with soft kernel texture in both F2 populations, plants with Gpc-B1 gene showed higher grain protein content than ones without Gpc-B1 gene. However, it was difficult to evaluate the effect of Gpc-B1 gene on yield and Japanese noodle quality with F2 plants. Now we are carrying marker-assisted backcross to produce near-isogenic lines to evaluate the effect of Gpc-B1 gene in Japanese soft wheat background more precisely.

**P243**

***In vitro* and *in vivo* studies of wheat storage proteins on dough quality in a model system**

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The aim of our work is, on one hand to study the role of the protein interactions during gluten formation and, on the other hand to study the effect of the wheat storage proteins on the dough quality by *in vitro* methods (dough reconstitution) and by *in vivo* transformation. To achieve this goal rice was used as a model system. Rice flour, due to the absence of wheat type storage proteins, could provide some advantages over wheat flour to use as base flour studying the characteristics of wheat prolamins. The methods developed for the characterization of wheat dough (micro-z-arm mixer) were adopted to determine mixing properties of the rice flour. A reduction/oxidation procedure was developed to incorporate glutenin subunit proteins into the polymeric structure of rice dough protein. Wheat gluten or its components, such as HMW and LMW wheat glutenin rich fractions, were incorporated in *in vitro* studies. Results show that the *in vitro* incorporated gluten has significant effect on functional properties of the rice dough. It was produced and analysed transgenic rice lines *in vivo* synthesizing substantial amounts of high-molecular-weight glutenin subunit (HMW-GS) from wheat. This alteration has considerable effect on the functional properties, including strength and stability of the dough made of transgenic rice flour. Our results demonstrate the potential of rice flour as a model system in wheat storage proteins structure-function studies, to determine the mechanism and the

specificity of disulphide bond formation between gluten proteins, the role of the protein interactions in wheat gluten formation and the contribution of certain proteins to quality attributes.

**P244**

**From cytogenetic to molecular approach and backwards: investigations of grain quality in bread wheat**

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The results of genetic investigations of grain quality will be presented which have been accomplished in the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia, since the late 1960s. Most of them were obtained using precise genetic stocks developed on the base of cultivars with contrasting grain quality, Saratovskaya 29 (S29, high quality) and Diamant 2 (Dm2, low quality). Monosomic and intervarietal substitution lines allowed identifying the chromosomes carrying the genetic factors responsible for separate technological parameters. In the last years with the use of ITMI recombinant inbred lines QTLs were detected on different chromosomes associated with grain and flour quality. The chromosomes are introducing now into the monosomic lines of S29 and Dm2 for verifying of the detected QTL. In parallel, S29/Janetzki Probat single chromosome recombinant double haploid lines for chromosomes involved in the determination of grain quality are being developed for mapping of genes associated with several technological characteristics. Another object of our interest is the set of bread wheat lines with small introgressions from wild relatives which enlarge the genetic polymorphism for the traits under study. At the present, the investigations are concentrated on search of the genetic factors regulating the folding of separate storage proteins into gluten and stabilizing its three-dimensional space structure.

**P245**

**Application of molecular markers, micro-level tests and interclass hybridizations in improving wheat grain quality**

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Wide spread use of within class hybridizations has reduced the genetic base of released varieties and restricted the combination of desirable alleles present in hard and soft wheat classes. In the present investigation the utility of combined use of molecular markers, micro-level tests and interclass hybridizations was assessed to improve wheat grain quality. 280 wheat varieties released in India during the last century, 192 soft and hard germplasm lines, 170 RILs and 300 back cross lines developed by interclass hybridizations were evaluated for grain hardness, sedimentation volume and grain protein content and molecular markers for puroindolines and 1B/1R translocation. The performance of genotypes over two year's period showed strong genetic factor influencing grain hardness ( $h^2 = 0.91$ ) and gluten strength ( $h^2 = 0.79$ ). Majority of Indian wheat varieties (90%) showed presence of null mutations in *puroindoline A* (*pinA*) and harder grain texture while all the identified soft germplasm lines exhibited wild alleles of both *pinA* and *pinB*. This demonstrated that selection pressure in Indian wheat breeding programme has been towards harder grain texture. This is because of harder grains are suitable for chapatti quality, the major form of wheat consumed in India. Both *pinA* and *pinB* were present in all the soft RILs and back cross lines while *pinA* was absent in all the

hard lines of these populations. Large variations were observed in sedimentation value in back cross populations developed using high yielding genotypes with 1B/1R translocation and soft germplasm lines. Presence of large numbers of transgressive segregants revealed that desirable alleles are present in both hard and soft wheat backgrounds. Therefore, the selection of genotypes using molecular markers and microlevel tests satisfying minimal requirements for preliminary indicators of quality e.g., kernel hardness, protein concentration and sedimentation volume appears practical. However, in later generations higher selection pressure for milling quality and actual baking process can be applied. Data validated the utility of interclass hybridizations, molecular markers and micro-level tests in wheat improvement because such hybridizations have not been explored widely in breeding programmes.

### P246

#### Characterisation of spelt (*Triticum aestivum* ssp. *spelta* L) germplasm for the polymorphism on the glutenin genes

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We evaluated 98 spelt accessions, collected from various parts of the world for their glutenin allele composition using the SDS-PAGE; and by MALDI-TOF. Glutenin subunit composition among the 98 lines showed large biodiversity, covering 64 different allelic combinations on the six loci. Based on the MALDI-TOF profiles, the population clustered into our distinct subgroups. At *Glu-A1*, *Glu-B1* and *Glu-D1*, encoding HMW-GS five, ten and six alleles were observed respectively. LMW-GS displayed similar polymorphism, as five alleles were identified at both the *Glu-A3* and *Glu-B3* respectively. Four alleles were observed at *Glu-D3* locus. Four of the 21 HMW-GS identified alleles were not present in any common bread wheats and can be characterised as specific for spelt. The level of polymorphism and the distribution of alleles at other loci, however seems to be similar than for common bread wheats, with one exception: The most frequent allele on *Glu-B1* is the 'f' allele (30.22%), followed by 'k' (26.53%) and 'b' (20.41). Results suggest that the genetic variation of the HMW and LMW glutenin alleles of spelt can be a rich source for improving spelt baking properties.

### P247

#### Mapping QTLs for quality characters in durum wheat (*Triticum turgidum* L. ssp. *durum*)

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In durum wheat, thousand-kernel weight (TKW), test weight (TW), grain protein content (GPC), gluten strength and yellow pigment content (YPC) are important traits for end use quality. A recombinant inbred line population developed from PDW 233 × Bhalegaon 4 was analyzed for these parameters across major durum growing environments in India and the molecular linkage map was developed to locate the QTLs responsible for variation in these traits. In composite interval mapping (CIM), five different QTLs associated with gluten strength, as measured by SDS-sedimentation

volume were identified. *Q<sub>Sv.macs-1B.1</sub>* flanked by marker interval *Xgwm550 - Glu-B3* was identified at LOD  $\geq 4.19$  in five environments and explained 9.18% to 40.66% of phenotypic variance of the trait. Along with glutenin coding loci *Glu-B1* and *Glu-B2* on 1B, loci on chromosomes 3B and 4B were also found to be associated with gluten strength. For YPC, five QTLs were identified on chromosomes 1A, 3B, 5B, 7A and 7B across five environments. The strongest one, *Q<sub>Yp.macs-7A</sub>*, located on the distal part of 7AL, explained 55.22% of the variation in the trait. Total seven QTLs, each accounting for 5.86% to 9.85% of variation in GPC, were identified, out of these, two each were detected on chromosomes 1B and 7A and one each on 2A, 5A and 7B. Three QTLs for TKW, each explaining 9.25% to 18.89% of variation, were located on chromosomes 2A, 4B and 6B, whereas, four QTLs influencing the TW with phenotypic variance ranging 7.78% - 11.58%, were detected on chromosomes 1A, 2A and 7A. QTLs identified for these important quality traits will be useful in marker assisted breeding for improvement of durum wheat.

### P248

#### Development of a codominant PCR-based marker for the wheat *Wx-B1* null allele

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Waxy protein is a key enzyme in the synthesis of amylose in endosperm tissue. The amylose content of wheat flour plays a significant role in determining Japanese udon noodle quality. Most wheat cultivars suitable for producing udon noodles have a low amylose level due to a lack of *Wx-B1* protein conditioned by the *Wx-B1* null allele. It was previously determined that the entire coding region of the wheat *Wx-B1* gene is deleted in the null allele. However, the extent and breakpoints of the deletion have not been established. In this study, the position of the 3' deletion breakpoint was refined by mapping with PCR-based markers. Using information from this analysis, a chromosome walk was initiated and the DNA sequence flanking the deletion breakpoints was obtained. The deletion included a 3872 bp region downstream from the termination codon of *Wx-B1* gene. Based on similarity with *T. monococum* sequences, it was estimated that approximately 60 kb upstream of the *Wx-B1* gene was also deleted. Using this sequence information, a codominant marker for the identification of the *Wx-B1* null allele was developed. This marker can unambiguously identify heterozygous plants, which will accelerate the selection of partial waxy mutants carrying the *Wx-B1* null allele.

### P249

#### Production of sweet wheat

**Shimbata T<sup>1</sup>, Saito M<sup>1,3</sup>, Takiya T<sup>1</sup>, Vrinten P<sup>2</sup>, Nakamura T<sup>3</sup>**

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The major components of storage starch are amylose and amylopectin, and in wheat, both an amylose-free mutant lacking granule-bound starch synthase I and a high-amylose mutant lacking starch synthase IIa have been produced recently. Here, we report the production of an amylose-free/ high-amylose double mutant. This double mutant has kernel and carbohydrate characteristics that are remarkably different than those of either single mutant, including a dramatically shrunken seed shape. Surprisingly, the double mutant has maltose and sucrose levels that are high enough to make it worthy of being called "sweet wheat".

**P250**

**Copying with wheat in Pakistan in the wake of green biotechnology, nanobiotechnology and food sovereignty**

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Wheat has remained the central theme of self sufficiency programme in Pakistan. Conventional wheat breeding has remained highly successful in Pakistan by sustaining and enhancing the so-called Green Revolution. Since 1963, when I first participated in the Second IWGS at Lund, Sweden, highly significant and far-reaching developments are constantly taking place in wheat sciences. In 2008, we are having very exciting time globally in genetic engineering and biotechnology when methods are constantly progressing to improve microarray quality control and to use transcription factors to discover unknown signal pathways. Simultaneously metabolomics has emerged as a significant player in the areas of genomics, proteomics, genetic engineering, biotechnology and nanobiotechnology. In 2008 numerous nanodevices and nanosystems for sequencing single molecules of DNA are feasible. Nanobiotechnology will play an important role in the study of systems biology-also referred to as pathway, network, or integrative biology-in which proteomics play an important role. Nanobiotechnology will provide refined tools for the study of genomics and proteomics, real time single particle tracing in living cells, and dissection of signaling pathways. As a supplementary approach to overall wheat improvement, green biotechnology should make a significant contribution. For facing the challenges of 21<sup>st</sup> century successfully it is necessary to identify and assess the frontiers not only in wheat biology (including green biotechnology) and agriculture but also in economics, sociology, ethics, international trade and politics in the wake of globalization, poverty alleviation and food sovereignty.

**P251**

**Development of durum wheat with varying percentage of B-starch granule content and relationship between starch swelling power and in-vitro starch digestion**

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Recent work has shown that changing the percentage of B-type starch granules in the starch of wheat can alter technological properties for baking (Park et al 2005), noodles (Chen et al 2003) and spaghetti made from durum wheat (Soh et al 2006). The latter study indicated there might be a benefit in developing durum wheats with slightly elevated B-granules for conventional pasta quality. This study describes the progress in the development of durum wheat germplasm with lower and elevated B-granule content compared to cultivated durum wheat (%B-granules ~22-27%). Particle size analysis of starches from 217 lines identified B-granule contents ranging from 19.9-43.3%. Selections were made for further crossing. Starch swelling power (SP) variation has been suggested as a useful test for developing wheats with potentially lower glycaemic index. We isolated starch from a set of wheat varieties that displayed variation in SP (8-16) and combined it with gluten and soluble components from durum wheat. The reconstituted flours that were obtained were made into spaghetti and the technological quality and in vitro starch hydrolysis rates were compared. Results will be discussed.

**P252**

**Wheat protein fractions in relation to grain quality characters of the cultivars registered in the Czech Republic 2004 - 2006**

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In the set of 15 winter wheat cultivars registered in the Czech Republic there were evaluated grain qualitative parameters with emphasis on protein fractions (content and proportion of albumins + globulins, content of gliadins and glutenins) plus relative viscosity. All parameters were significantly influenced by year differences. Strong influence of genotype was observed in proportion of albumins + globulins in crude protein and content of this protein fraction in dry matter. Also content of albumins + globulins in dry matter and gluten index (GI) were influenced by genotype. Cultivar Rialto showed the highest content (3.93% in d.m.) and proportion (30.81 %) of albumins + globulins. The highest value of relative viscosity which can potentially deteriorate feeding value of wheat grain for poultry was observed in cultivars Sulamit (2.50) and Rialto (2.11). Higher negative correlation was confirmed between proportion of albumins + globulins in crude protein on one side and storage protein fractions gliadins, glutenins on the other side ( $r = -0.54; -0.69$  respectively).

**P253**

**Comparison of the effects of Puroindoline Genotypes on Grain and Flour Properties Using Near Isogenic Lines**

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Grain texture is one of the most important characteristics for end-use quality of wheat. The *Ha* (Hardness) locus located on the short arm of chromosome 5D is known to control grain hardness. This locus encodes 15 kDa protein called friabilin which consists of puroindoline a (*Pina*), puroindoline b (*Pinb*) and grain softness protein 1 (*Gsp-1*) genes. Both expression of *Pina* and *Pinb* gene is soft grain. The mutations of *Pina* or *Pinb* genes were associated with hard texture. Some mutations of *Pina* and *Pinb* have been reported varying grain hardness in hard wheat. We studied the effect of puroindoline alleles on the grain hardness, milling and flour properties. We developed near iso-genic lines of five kinds of *Pina* or *Pinb* genes; *Pina-D1b*, *Pinb-D1b*, *Pinb-D1c*, *Pinb-D1p* and *Pina-D1k/Pinb-D1q* (double null), in soft wheat cultivar Fukuhonoka. NILs and Fukuhonoka were cultivated with three replicates at WeNARC. The grain characteristics (weight, diameter and hardness) were measured with an SKCS 4100. Grain protein content was measured with near-infrared spectroscopy. Grain samples were milled on a Brabender Jr. test mill. Flour characteristics measured the flour particle size distribution, SDS-sedimentation test and damaged starch. The different effects were observed on the grain hardness, flour particle size and damaged starch content among them. The effect of puroindoline genes to those quality traits was estimated as follows; *Pina-D1k/Pinb-D1q*  $\geq$  *Pina-D1b*  $>$  *Pinb-D1c* = *Pinb-D1p*  $>$  *Pinb-D1b*.

**P254**

**Improvement of dough strength for bread-making quality in Japanese common wheat**

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Many cultivars in Europe and America showing good bread-making characters contain an allele of the high-molecular-weight glutenin subunit, *Glu-D1d*, located on chromosome 1D. We introduced this

allele into four Japanese leading cultivars with low bread-making quality by the recurrent backcrossing method and produced near isogenic lines. The dough of these lines with *Glu-D1d* was stronger than the recipient cultivars. However, the strength did not reach the level of the donor cultivar, ‘Haruhikari’, with a good bread-making quality. To reveal the other factors of ‘Haruhikari’ on dough strength, we analyzed the effect of low-molecular-weight glutenin subunit (LMW-GS) using locus-specific primers of LMW-GS genes and gliadin bands tightly linked to LMW-GS genes. Segregation analysis of the F<sub>2</sub> between ‘Haruhikari’ and ‘Asakaze-komugi’ with a poor bread-making quality revealed that an amplified LMW-GS gene involved in the multigenes on *Glu-B3* of ‘Haruhikari’ had the significant effect on dough strength. The gene of ‘Haruhikari’, comparing to ‘Asakaze-komugi’, had a seven amino-acid deletion in the repetitive domain and three amino-acid substitutions, changing the hydrophilicity. The presence of the gene and the other genes on *Glu-B3* tightly linked with it must affect the dough strength.

### P255

#### Breeding and introduction of waxy wheat Nuomai No.12

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Waxy wheat contains <1% amylose in the endosperm starch. This research has utilized waxy wheat (the progeny of Baihuomai×Kanto107) and main cultivated varieties in South China as parents. Lasting for 5 years and 10 seasons, by means of molecular marker-assisted selection, combine limited backcross (cultivated varieties in South China as recurrent parents), summer multiplication as well as the assistance of biochemistry molecular marker selection, we have developed some special waxy wheats, such as: Nuomai No.12. The starch in the endosperm of Nuomai No.12 contains 100% amylopectin (Amylose-free). This special wheat line has integrated the good characters of waxy starch, high yield and widely adaptability. The parameters of Peak Viscosity, Trough Viscosity and Final Viscosity in Nuomai No.12 are 36.4; 8.3; 12.7 RVU respectively. Nuomai12 has abundance of mineral elements such as Fe, Na, Mg, K; The seed of Nuomai No.12 is rich in pentosan, a healthful ingredient for human body. In addition, Nuomai No.12 has possessed good agronomy character and nature of strong springiness and satisfying early maturity (special early maturity, 10 days earlier than the common cultivars). The yield of Nuomai No.12 equals the current cultivars named Chuanyu 12 which with high yield in South China. The rice glue ball made of Nuomai No.12 is nutritious and smooth to the taste while the soup is not muddy. All the good characters above could ensure Nuomai No.12 a wide market in the future.

### P256

#### Proteins identification of wheat-rye translocation lines by MALDI-TOF-TOF mass spectrometry and ESI-QTOF/MS

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The wheat-rye translocation lines have been agriculturally developed for the resistant crop to several pathogens and drought stress. The Hamlet-line was obtained by crossing between wheat cultivar ND7532 and rye cultivar Chaupon to translocate the chromosome 2RL (*H21* resistant gene to biotype

L of Hessian fly) from rye to wheat. In order to identify 2RL chromosome-derived specific proteins, we compared the proteome of ‘Coker797’ (non-2RL) with those of ‘Hamlet’ (2RL) and near-isogenic line (NIL) carrying 2RL by 2D-gel electrophoresis and MALDI-TOF<sup>2</sup>/ESI-qTOF-MS. The proteins from leaves of three lines were extracted by trichloroacetic acid/acetone precipitation method. In the proteomic analysis, 24 protein spots were clearly increased in 2RL-carrying lines compared to non-2RL line. From the selected spots, 27 proteins in total were putatively identified by tandem mass spectrometry, which were corresponded to 18 unique proteins. Interestingly, heat shock protein 70, chaperon protein DnaK, malate dehydrogenase I, and triosephosphate isomerase were confirmed in the previous expressed sequence tag database of NILs cDNA library. In additions, methionine synthase, ATP synthase CF1 alpha/beta chain, Rubisco large subunit, NADPH dehydrogenase, ferredoxin-NADP(H) oxidoreductase, resistance protein, and plastid-lipid associated proteins were exclusively identified in 2RL-lines by proteomic approach. The physiological function of newly identified proteins is under investigation. Therefore, the in-depth proteomic approach combined with genomics will help catalogue the actually expressing proteins responsible for the resistance to biotic and abiotic stress.

### P257

#### Chromosome location and characterization of genes for grain protein content in *Triticum dicoccoides*

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Three sets of durum Langdon-*Triticum dicoccoides* (LDN-DIC) disomic chromosome substitution lines were previously developed by L. R. Joppa using *T. dicoccoides* accessions Israel A, PI 481521, and PI 478742 as the chromosome donors and durum Langdon (LDN) as the recipient. The set based on Israel A was well characterized previously, resulting in the identification and isolation of *Gpc-B1*, a major gene for high grain protein content (GPC) on chromosome 6B. This study was conducted to identify and characterize genes for high GPC in the two sets based on PI 481521 and PI 478742. The two sets of (LDN-PI 481521 and LDN-PI 478742) substitutions lines and controls were grown at Fargo and Prosper in North Dakota in 2005 and 2006 using a randomized complete block design with four replications. The GPC analysis from the trials showed that the substitution lines LDN(PI 478742-6B) and LDN(PI 481521-7B) had the highest GPC within their respective sets, suggesting that chromosome 6B of PI 478742 and 7B of PI 481521 carry high GPC genes. In addition, five other substitutions including 1A, 2A, and 5B of PI 481521 and 7A and 5B of PI 478742 had significantly higher GPC than LDN. To determine if the GPC gene in PI 478742 is the same as *Gpc-B1*, we screened three *T. dicoccoides* accessions and their 6B substitution lines using an allele specific marker *Xuhw89* for *Gpc-B1*. Marker analysis indicated that chromosome 6B of PI 478742 carried the same *Gpc-B1* allele as Israel A; however PI 481521 had the wild type allele found in LDN. A comparison with previously reported GPC genes in wheat and related species suggested that chromosomes 2A and 7B of PI 481521 and 7A of PI 478742 are likely candidates as sources of new high GPC genes.

### P258

#### Addition of chromosome 5H of *Hordeum* species to wheat enhances grain softness

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Grain hardness is an important factor affecting end-use quality in wheat. Puroindoline a (PINA) and puroindoline b (PINB) protein are the major determinants of grain hardness and the genes for PINA and PINB are located on chromosome 5DS. Hordoindolines are the puroindoline homologs located on chromosome 5HS in barley. In this study, we used three sets of wheat-*Hordeum* species (*H. vulgare*,

*H. vulgare ssp. spontaneum*, and *H. chilense*) chromosome addition lines. We studied the effect of each chromosome of *Hordeum* species on wheat grain hardness. Grain characteristics were measured by single kernel characterization system (SKCS). The addition lines of chromosome 5H resulted in significantly lower SKCS hardness index than the corresponding wheat parents except that of *H. vulgare*. Although significant difference was not found, the addition line of chromosome 5H of *H. vulgare* showed lower hardness index than the wheat parent. All of the addition lines of chromosome 5H showed significantly higher 1000-kernel weight than the corresponding wheat parent. While the degree of increase of 1000-kernel weight was similar among wheat-barley 5H addition lines, the effect of enhancing grain softness was largest in wheat-*H. chilense* addition line. These results indicated that chromosome 5H of *Hordeum* species increased grain softness and kernel weight in the genetic background of wheat and the effect on grain hardness seemed to depend on *Hordeum* species. The barley hordoin or other proteins on chromosome 5H may play a role in reducing grain hardness.

### P259

#### Establishment of multiplex PCR system for high molecular weight glutenin subunits in wheat

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Wheat processing quality is closely correlated with composition and quantity of glutenin, in particular with the high-molecular weight glutenin subunits (HMW-GS) encoded by the *Glu-1* locus. The specific molecular markers of the subunits correlated with good processing quality such as 1Dx5, 1Ax2\* and 1Bx7<sup>OE</sup> have been developed and been applied to molecular breeding of quality improving. The purpose of this study was to establish the multiplex PCR system to simultaneously identify 1Ax2\*, 1Bx7<sup>OE</sup> and 1Dx5 subunits in one reaction through exploring the influences of PCR components and cyclic parameters to the multiplex PCR results. The results showed that the primers concentration ratio and T<sub>m</sub> value are the critical factors to the success of multiplex PCR, when the primers concentration ratio was 1Dx5/1Bx7<sup>OE</sup>/1Ax2\*=0.1/0.2/0.3 or 0.1/0.2/0.4 and T<sub>m</sub>=60, the outcome of multiplex PCR of 1Ax2\*, 1Bx7<sup>OE</sup> and 1Dx5 was the best. The multiplex PCR system can identify more than one HMW-GS quickly and efficiently simultaneously in one reaction, can be used to carry out multiplex molecular marker assisted selection in wheat quality breeding.

### P260

#### Utilization of near isogenic lines of gliadin loci of common wheat for comparison of three existing A-PAGE methods of gliadin analysis

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Gliadins are alcohol-soluble seed storage proteins with high level of intervarietal polymorphism, which is generally evaluated by methods of acid electrophoresis in acryl amide gel (A-PAGE). Expression of the most gliadin bands controlled by six loci, located on homoeologous group 1 (*Gli-1*) and 6 (*Gli-2*) chromosomes. In a number of works it was shown their influence on bread-making quality of wheat as well as it was found out their links with particular agronomic important traits. Very extensively gliadins used for investigation of intervarietal polymorphism and for varieties identification. Currently there are known a few A-PAGE methods for gliadins separation. The A-PAGE method proposed by Zillman and Bushuk (1979) based on separation of proteins in Al-lactic buffer system without addition of urea in gel. For this method the international catalogue of gliadin

alleles was developed by Metakovsky (1991). This method was applied for investigations in a number of laboratories worldwide. At the Eastern European countries and particular in countries of former Soviet Union much more extensively is used A-PAGE method based on separation of gliadins in glycine-acetate buffer system in gel containing up to 8M of urea, for which another catalogue of alleles was developed (Sobko, Poperelya 1986; Poperelya, 1996). The third method proposed by Brzezinski and based on separation of gliadins in buffer system of formic acid for which the catalogue was not developed. In order to construct the matching system for the allele identification obtained by utilization of different A-PAGE methods we use the set of near isogenic lines of gliadin loci developed by Kopus (1994) on background of cv. Bezostaya 1. Those lines carry alleles the most common for world winter bread wheat gene pool. Our data will be very helpful for comparison of results on gliadin analysis obtained by different methods and performed in different laboratories as well as will make the basis for development the catalogue of gliadin allele for A-PAGE method based on formic buffer system.

### **P261**

#### **Tendencies in baking quality of common wheat varieties realised in Ukraine and their influence on allele frequency of storage protein genes**

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Breeding for high bread making quality as well as registration of varieties with such characteristics ever if they were developed in other countries is the main task in Ukrainian grain business in resent 20-30 years. We are going to present the main tendencies in bread-making quality characteristics which we observed during two last decades of monitoring of winter common wheat varieties realised in Ukraine and the main features in allele frequencies of the genes, which have the key influence on development of this trait in wheat. This investigation was performed in order to reveal the efficiency of application of particular markers in breeding for incretion of bread-making quality. It was analysed three main genetic systems high molecular weight glutenins, gliadins and puroindolines. It was shown the low level of polymorphism in alleles controlling by *Glu-1* loci if compare to world known alleles. In *Glu-A1* locus it was detected alleles *a* and *b* in high frequency. In *Glu-B1* locus alleles *b* and *c* were mainly found out, then *d* and only one variety possesses *e* allele. Resent two years in a few cultivars was identified allele *al Glu-B1*, which perform the over expression unit Bx7 and was not found out resent 20-30 years in Ukrainian common wheat gene pool. In *Glu-D1* locus allele *d* mainly was shown. In gliadin alleles it was observed the combination of “old” alleles, traditionally presented in Eastern European gene pool as well as “new” one, which previously was not identified. The frequencies of those alleles are different in each particular group of quality (strong, valuable, filler) as well as “new” alleles marked loci with both positive and negative effect on bread-making quality characteristics. Conservatism was evaluated in *pinA* and *pinB* alleles. Analysis of our results sowed the bottleneck of particular alleles i.e. loci compared to worldwide distribution as well as suggestion that ever those three main genetic systems are not able to predict totally the end-use quality of breads wheat.