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The application of new tools to study gene expression and genetic diversity in allohexaploid wheat

Edwards KJ, Coghill JA, Poole R, Winfield MO & Barker GL
School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG

We have used the Affymetrix wheat GeneChip to characterise aspects of wheat development. In addition, we have used standard PCR-based technologies to genotype various wheat collections. Unfortunately, all microarray and genotyping platforms are designed for diploid species and their use in polyploids creates unique problems. For instance, we have recently reported that current transcriptome-based assays, including q-RT-PCR, are often unable to discriminate between related homoeologous transcripts. As such, these platforms at best report the sum of the expression of related transcripts. Likewise, most genotyping platforms are unable to discriminate between related homoeologous sequences; a situation that has resulted in high-throughput genotyping in wheat being confined to random sequences selected on the bases of their copy number rather than their usefulness to wheat breeders.

To investigate these polyploid induced problems, we have examined the possibility of using the available wheat sequences to target specific genes for use either in homoeologous specific microarrays or in multiplexed genotyping platforms.

For our transcriptomics-based studies, we have used a modification of the AutoSNP software to design a 244,000-feature Agilent array containing homoeologous and paralogous specific oligonucleotides representing >10,000 sequence clusters. Our preliminary analysis of the data generated from this array suggests that while data analysis is complicated, such arrays provide new information on the transcriptome of both wheat and its progenitor species.

For the genotyping platform, we have focused our efforts on using SNPs from both agronomically important genes and randomly selected ESTs derived from the wheat community. We have used non-amplified genomic DNA and padlock probe pairs to differentiate between similar sequences in the wheat genome. Our results suggest that padlock probes are capable of discriminating between homoeologous sequences and hence can be used to efficiently genotype wheat varieties.

However, both of the above studies strongly suggest that to be efficient at discriminating between homoeologs, probes need to be designed to a precise format. Unfortunately, this design precludes the use of most sequences in such studies. This aspect of wheat genetics will be discussed during my talk and I will put forward, what I think, is the only viable solution to effective transcriptome and genome analysis of polyploid wheat.

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AMMI analysis for stability and location effect on grain yield and its components of durum wheat genotypes

Haile JK[13], Sarial AK[12], Assefa S[3], Sareen S[4]
[1]*Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia*
[2] *COA, Haryana Agricultural University Campus, Kaul, 136021 India*
[3]*Ethipian Institute of Agricultural Research, Debre-Zeit, Ethiopia*
[4]*Directorate of Wheat Research, ICAR, Karnal-India*

Developing crop cultivars with high grain yield has been the principal aim of durum wheat breeding programs worldwide. In Ethiopia, it is of special interest because of the low and erratic distribution of rainfall. Understanding the effect of environmental variations on yield and its components becomes an essential step in the development of high-yielding and more stable cultivars. In this context, an experiment was conducted in twelve environments comprising of six locations with two planting patterns (row and broadcasting) to evaluate 25 genotypes of durum wheat of which 14 were introduced from CIMMYT for stability of grain yield and its components. The genotypes were grown in 2004 growing season in a RBD with three replications in 2 m^2 plot size. Stability analysis was done as per Eberhart & Russel (1966) model and its interaction with genotypes were estimated following additive main effects and multiplicative interaction (AMMI) model. The G x E interaction was highly significant for grain yield/plant, biological yield/plant, number of grains/spike and 1000 kernel weight. This has been a challenge to durum wheat breeders as these interactions limit accuracy of yield estimate, identification of stable genotypes and further complicates selection of genotypes even for specific environments. Both stability analyses proved that genotypes 'CD97383', ' CIGM91-349' and 'DZ3117' exhibited more stable performance compared to others for grain yield. The environments showed high variability both in mean yield and interaction pattern, but Debre Zeit and Akaki were found to be more favourable for all genotypes. Alem Tena was favorable environment for production of durum wheat genotypes with high protein content.

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Wheat (*Triticum aestivum* L.) root proteome and identification of differentially expressed proteins between hybrid and parents

Sun Qixin, Song Xiao, Ni Zhongfu, Yao Yingyin, Zhang Yinhong
Department of Plant Genetics and Breeding, China Agricultural University, Beijing, 100094, China

To gain a better understanding of the molecular basis of wheat heterosis, we carried out a comparative proteomic analysis in seedling leaves and roots between wheat hybrid and parents. One heterotic F_1 hybrid and its parents were used for comparative proteomic analysis. Firstly, a reference map of the major soluble proteins of wheat roots and leaves were established using a combination of 2-DE and MALDI TOF MS and MS/MS, and a total of 450 protein spots were detected with silver staining in a pH ranges of 4 to 7, of which 282 spots corresponding to 240 proteins were identified. A total of 45 differentially expressed protein spots were detected, and both quantitative and qualitative differences could be observed. Moreover, 25 of the 45 differentially expressed protein spots were identified, which were involved in diverse pathways. The expression patterns of the total proteins in seedling leaves were also compared between hybrid and its parent by using two-dimensional gel electrophoresis with two pH ranges for the first dimension separation. Among ~900 protein spots reproducibly detected in leaves, forty-nine protein spots were identified as being differentially expressed between hybrid and its parental lines ($P < 0.05$) for more than 1.5 folds. Six possible modes

of differential expression were observed, including high- and low-parent dominance, underdominance, and overdominance, uniparent silencing and uniparent dominance. Moreover, 30 of the 49 differentially expressed protein spots were identified, which were involved in metabolism, signal transduction, energy, cell growth & division, disease & defense, secondary metabolism. These results indicated that hybridization between two parental lines can cause expression differences between wheat hybrid and its parents not only at mRNA levels but also at protein abundances, and the proteins differentially accumulated between hybrids and their parents were involved in diverse physiological process pathways, which might be responsible for the observed heterosis.

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Genes and gene networks regulating wheat development

Dubcovsky J [1], Li C [1], Distelfeld A [1], Pidal B [2], Tranquilli G [2]
[1]Plant Sciences Department, Univ. of California, Davis CA 95616, USA
[2]Instituto de Recursos Biológicos, INTA, Buenos Aires, Argentina

A correct flowering time is critical to optimize seed production. Therefore, plants exhibit a complex regulation of the transition between vegetative and reproductive shoot apical meristems. We positionally cloned three genes that regulate this transition in wheat in response to vernalization (the exposure to cold temperatures for an extended period of time). The most critical one is *Vrn-1* (a homologue of Arabidopsis *AP1*) since its absence results in plants that never flower. *Vrn-1* is regulated by *Vrn-2* (a cereal specific CCT transcription factor that represses *Vrn-1*) and *Vrn-3* (a homologue of Arabidopsis FT, which promotes flowering). The interactions between these genes and *Vrn-1* are likely mediated by intermediary proteins. VRN-3 interacts with the bZIP protein FDL2 that binds *in vitro* to four elements (core sequence ACGT) present in the *Vrn-1* promoter. Shift mobility and ChIP experiments failed to show a direct interaction between VRN-2 and *Vrn-1* regulatory regions suggesting an indirect interaction. We recently found a diploid wheat accession that carries a 48-bp deletion including the complete CArG box and still exhibits a winter growth habit. This result indicates that the CArG box located in the *Vrn-1* promoter is not essential for the *Vrn-2* mediated repression of *Vrn-1*. Epistatic interactions between *Vrn-2* and different dominant alleles of *Vrn-1* suggest that *Vrn-2* interacts mainly with a region within *Vrn-1* first intron and possible with a promoter region upstream of the CArG box. We found that this CArG box is the binding site for TaVRT2, a MADS-box gene upregulated by vernalization independently of *Vrn-1*.The central role of *Vrn-2* in the determination of the winter growth habit in tetraploid wheat was confirmed by generating a double recessive *vrn-A2 vrn-B2* line. This line showed a spring growth habit in spite of the presence of homozygous *vrn-1* alleles for winter growth habit. This double recessive line was used to discover functional and non-functional copies of *Vrn-A2* and *Vrn-B2* in polyploid wheat. We will discuss a model describing our current knowledge on the regulation of vernalization response in polyploid wheat.

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Integration of low-temperature and long-day flowering responses in cereals

Trevaskis B, Hemming MH, Dennis ES, Peacock WJ
Commonwealth Scientific and Industrial Research Organisation, Division of Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia

The temperate cereals barley and wheat perceive seasonal environmental cues to synchronise flowering with optimal conditions in spring. Two important cues are prolonged exposure to low temperature over winter (vernalization) and long-daylengths. The requirement for exposure to low-temperature overrides the effect of long-days, and varieties with a strong vernalization requirement

only flower in response to long-days after vernalization has occurred. This integration of low-temperature and daylength responses ensures that flowering does not occur until spring. By examining genetic interactions between three genes that control the vernalization requirement in temperate cereals – *VRN1*, *VRN2* and *FT1* (*VRN3*), we have been able to describe how low-temperature and long-day responses are integrated. The low-temperature response is mediated by *VRN1*, a promoter of flowering that is normally activated by vernalization during winter. The daylength response is controlled by *FT1*, which is induced by long-days. *VRN2* plays a central role in integrating the low-temperature and long-day responses by maintaining low levels of *FT1* expression and suppressing the long-day response in plants that have not been vernalized. Following vernalization, *VRN1* is expressed and represses *VRN2* to allow long-day induction of *FT*. According to this model, natural variation in vernalization requirement has been achieved through different mechanisms: mutations that activate *VRN1* substitute for low-temperature treatment, whereas loss of function mutations in *VRN2*, or mutations that increase *FT1* (*VRN3*) expression, activate the long-day flowering response without prior vernalization. We will present data to support our model of the integration of floral pathways in cereals and discuss how a better understanding of the genetic interactions between *VRN1*, *VRN2* and *FT1* will be useful in cereal breeding strategies.