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

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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.