WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND DNA-PROFILING IN PARTICULAR

Thirteenth Session
Brasilia, November 22 to 24, 2011

ADDENDUM

DEMONSTRATION OF SIGNIFICANT PROGRESS TOWARDS AN OPTION 1 APPROACH IN BARLEY

Document prepared by experts from the United Kingdom
Plant Science into practice

SNPs for barley DUS assessment:
James Cockram and Donal O’Sullivan

Advances in understanding the molecular basis for variation in barley characteristics:

A project funded by Fera

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Advances in understanding the molecular basis for variation in barley characteristics

- Association Genetics of UK Elite Barley (AGOUEB), BBSRC LINK project
- ~500 barley varieties genotyped
- 1536 SNP loci
- Association mapping used to detect associations between SNPs and DUS characteristics

Objectives

- Identification of relevant genetic loci
  - 28 DUS characteristics (some VCU characteristics also used)
  - Literature searches showed genetic loci mapped for 12 of the 28 DUS characteristics
- Identification of relevant genetic markers
  - Genotype assays designed for each selected gene
- Genetic marker validation
  - 90 European barley varieties (malting, feed, 2/6 row, winter/spring)
- Interpretation of DUS marker genotypes
  - Predictive value of genetic markers for the relevant characteristic assessed
The KASPar platform

Visualisation of SNP data generated from assay HvOs03g14250_C82T (kernel: colour of aleurone layer), using SNP Viewer (KBiosciences). The alternative SNP genotypes are clearly distinguishable (T:T = red, C:C = blue), with heterozygous individuals (T:C = green) unambiguously clustered in a separate cloud. The water negative control is shown in black, while unknown calls (predicted to represent wells which lack DNA) are shown in pink.

Relevance to DUS testing and objectives

• Developments in the genetic understanding of DUS characteristics are now being matched by advances in cheaper genotyping platforms
• Project aimed to produce a rapid marker test for as many DUS barley characteristics as possible
• Assess linked and putatively causative SNPs for their ability to predict DUS characteristic states
Genetic marker validation

• 82 assays designed and converted to the KASpar platform
• 3 did not work and 20 were unreliable – this was due to insufficient separation between allele clusters, or inability of the KASpar platform to convert assays testing for Indel genetic polymorphisms
• 57 reliable assays had a missing score rate of 1% (a high genotyping success rate)

Genotyping validated markers in UK germplasm

• 169 UK barley varieties were genotyped with the 57 validated KASPar assays and compared to a database of phenotypic data
• Predictive values were based on the percentage of correctly called characteristic scores
• Predictive values varied widely, with highest values obtained from markers originating from cloned genes
Group 1: Characteristics which provide perfect (100%) prediction by markers

- Ear: number of rows
- Grain: disposition of lodicules
- Seasonal growth type

Group 2: Characteristics which provide very good (>90%) prediction by markers

- Kernel: colour of aleurone layer
- Lower leaves: hairiness of leaf sheaths
**Group 2: Characteristics which provide good (>80%) prediction by markers**

- Sterile spikelet: attitude
- Grain: ventral furrow – presence of hairs

**Prediction of phenotypes from markers**

- Highest values obtained from markers originating from cloned genes (seasonal growth type)
- Although a marker prediction score of 100% was observed for “Grain: disposition of lodicules”, only one variety in the UK panel had ‘bib’ type phenotype
- Markers for “Lower leaf: hairiness of leaf sheaths” gave a 96% predictive value
- This characteristic is difficult to score in the field and is a good candidate for map-based cloning
- For the anthocyanin based characteristics (3, 7 and 23), markers were able to predict presence or absence of anthocyanin, but not intensity
## Predictive values of genetic markers for DUS characteristics

<table>
<thead>
<tr>
<th>Trait</th>
<th>UPOV No.</th>
<th>Marker</th>
<th>Chr</th>
<th>Vars</th>
<th>Geno &amp; Pheno No.</th>
<th>Pred correct</th>
<th>Pred %</th>
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</table>

### Positives.....

- 13 assays give perfect or very good predictions of DUS characteristics
- KASPar assays could be supplemented by some agarose-based INDEL analysis
- Information could be used to populate a molecular database
- Similar varieties could be grouped in the field
- Group 1 characteristics with 100% prediction (Seasonal growth type; Grain:disposition of lodicules; Ear: number of rows) could potentially be replaced by genotyping
- Cost of genotyping is low
Negatives.....

- Anything with less than 100% prediction is not as good as phenotyping!
- Uniformity not currently assessed
- Replacing field assessment with assays for two of the characteristics from Group 1 would be of no immediate benefit:
  - Ear: number of rows, quick and easy assessment in the field
  - Grain: disposition of lodicules, difficult to assess but only one variety found with 'b6' type lodicules!
  - Seasonal growth type was assessed separately in another project reported at the 2010 BMT

Conclusions

- Considerable progress has been made towards the understanding of the genetic control of DUS characteristics in barley
- Assays have been developed to predict DUS characteristics with high success rates
- Although 100% success rate was achieved in some characteristics, this is needed in all characteristics if phenotypic assessment is to be replaced
- Uniformity assessment needs to be addressed before implementation of a molecular assay for DUS characteristics
- Marker assays are currently not as good as the field assessment!