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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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

National Institute of Agricultural Botany

Presenter Name Carol Norris Date November 2011

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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SNPs for barley DUS assessment:

James Cockram and Donal O'Sullivan

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

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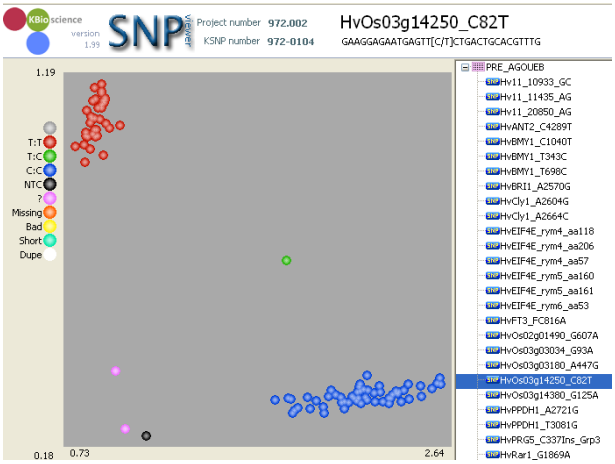
Objectives

- 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.



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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)

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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

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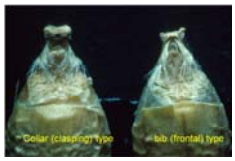
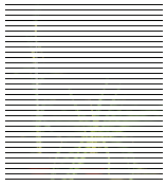
Group 1: Characteristics which provide perfect (100%) prediction by markers

- Ear: number of rows

- Grain: disposition of lodicules



- Seasonal growth type



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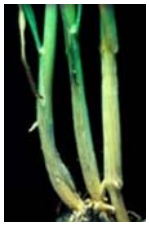
Group 2: Characteristics which provide very good (>90%) prediction by markers

- 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

Trait	UPOV No.	Marker	Chr	No. Vars Geno & Pheno	No. correct pred	% correct pred
Growth habit	1	HvFT3_FC816A	1H	122	107	**87.7**
Lower leaves: hairiness of leaf sheaths	2G	HvOs03g03180_A447G	4H	158	151	**95.0**
Lower leaves: hairiness of leaf sheaths	2G	HvOs03g03034_G93A	4H	158	148	**93.1**
Lower leaves: hairiness of leaf sheaths	2G	Hv11_11299_GC	4H	156	151	**96.2**
Lower leaves: hairiness of leaf sheaths	2G	Hv11_20007_GA	4H	155	140	90.3
Flag leaf: intensity of anthocyanin colouration of auricles [2]	3	HvANT2_C4289T	2H	146	144	**98.0**
Awns: intensity of anthocyanin colouration of awn tips [2]	7	HvANT2_C4289T	2H	148	145	**97.3**
Grain: anthocyanin colouration of lemma nerves [2]	23	HvANT2_C4289T	2H	153	142	**92.2**
Ear: number of rows [3]	11G	HvVRS1_C349G	2H	160	152	**94.4**
Ear: number of rows [3]	11G	HvVRS1_GINS681	2H	160	145	90.1
Ear: number of rows [4]	11G	HvVRS1_C349G & HvVRS1_GINS681	2H	159	152	**95.0**
Ear: number of rows [5]	11G	Hv11_20606_GC	4H	157	157	**99.4**
Sterile spikelet: attitude (mid 1/3 of ear) [6]	19	Hv11_10933_GC	1H	128	113	87.6
Sterile spikelet: attitude (mid 1/3 of ear) [6]	19	Hv11_11359_GC	1H	127	111	86.7
Sterile spikelet: attitude (mid 1/3 of ear) [6]	19	Hv11_21333_CG	1H	128	110	85.3
Grain: rachilla hair type	21	Hv11_20449_TA	5H	161	79	48.8
Grain: rachilla hair type	21	Hv11_10622_GA	5H	152	104	68.0
Grain: rachilla hair type	21	Hv11_20850_AG	5H	160	111	68.9
Grain: spiculation of inner lateral nerves [7]	24	Hv11_10818_CA	2H	157	92	58.2
Grain: spiculation of inner lateral nerves [8]	24	Hv11_11435_AG	2H	158	92	57.9
Grain: ventral furrow - presence of hairs	25G	HvOs02g01490_G607A	6H	161	132	81.5
Grain: ventral furrow - presence of hairs	25G	Hv11_21204_GA	6H	160	114	70.8
Grain: disposition of lodicules [9]	26	HvCly1_A2604G	2H	155	155	**100**
Grain: disposition of lodicules [10]	26	HvCly1_A2664C	2H	156	155	**98.7**
Kernel: colour of aleurone layer [11]	27	HvOs03g14250_C82T	4H	157	135	85.4
Kernel: colour of aleurone layer [12]	27	HvOs03g14380_G125A	4H	158	146	**92.4**
Kernel: colour of aleurone layer [13]	27	Hv11_21296_CA	4H	155	143	91.7
Seasonal growth habit [14]	28G	VRN-H1 Multiplex PCR	5H	143	143	**100.0**
Seasonal growth habit [15]	28G	HvVRNH1_SNP2	5H	137	129	**94.2**
Seasonal growth habit [16]	28G	HvVRNH1_SNP2 & HvVRNH1_hap2_InDel	5H	135	134	**99.3**

Predictive values of genetic markers for DUS characteristics

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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 'bib' type lodicules!
  - Seasonal growth type was assessed separately in another project reported at the 2010 BMT

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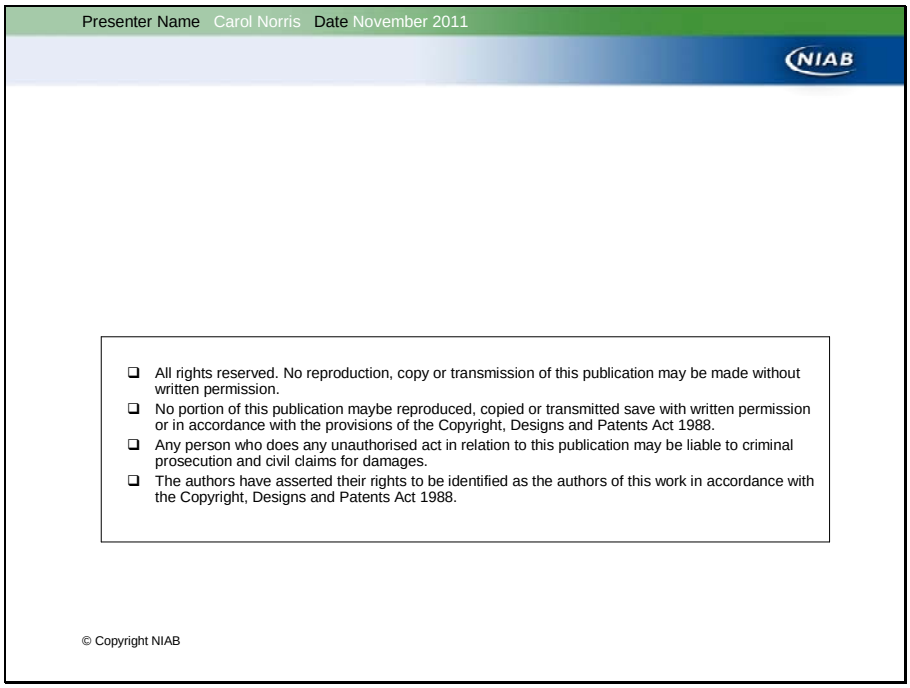
Conclusions

- 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!



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