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DEMONSTRATION OF SIGNIFICANT PROGRESS TOWARDS AN OPTION 1 APPROACH IN BARLEY

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DEMONSTRATION OF SIGNIFICANT PROGRESS TOWARDS AN OPTION 1 APPROACH IN BARLEY

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1. INTRODUCTION

Advances in understanding the molecular basis for variation in barley DUS traits

1. Recently, as part of a BBSRC LINK project – Association Genetics of UK Elite Barley (AGOUEB) – in which barley varieties which were National Listed from 1993 to 2005 were genotyped at 1536 SNP loci, we have used the technique of association mapping to detect associations between individual single nucleotide polymorphisms (SNPs) and a number of DUS characteristics. Eight DUS characteristics (Grain: rachilla hair type (UPOV Test Guidelines: TG/19/10, Char. 22); Lowest leaves: hairiness of leaf sheath (UPOV Char. 2); Grain: hairiness of ventral furrow hairs (UPOV Char. 26); Grain: spiculation of inner lateral nerves of dorsal side of lemma (UPOV Char. 25); Flag leaf: anthocyanin coloration of auricles (UPOV Chars. 3, 4); Awns: anthocyanin coloration of tips (UPOV Chars. 8, 9); Kernel: color of aleurone layer (UPOV Char. 28); Sterile spikelet: attitude (UPOV Char. 20)) have been mapped at approx. 3 cM resolution with individual SNPs showing a very high degree of correlation between allelic state of the SNP locus and characteristic state. We are currently assessing candidate genes in the immediate vicinity of these eight characteristic association peaks for 'causative' polymorphisms rather than 'linked' SNPs.

2. The opportunity therefore now exists to rapidly assess the currently available suite of linked and putatively causative SNPs for their ability to predict individual characteristic states. Potentially, in addition to seasonal type and ear row number, eight additional DUS characteristics, including some which are not trivial to score manually, can be streamlined into a rapid marker test. To maximize the potential of the assay, functional polymorphisms which tag alleles of cloned barley resistance genes and some value for cultivation and use (VCU)-type quality traits will be included in the study. For the first time in a major arable crop species, it is possible to constitute a collection of DNA markers which assay for proven functional polymorphisms in genes underlying variation in DUS characteristics. We will assemble a panel of up to 96 marker assays either diagnostic for known functional alleles in DUS characteristics or VCU traits or tightly linked to, and therefore predictive of, phenotypic status of a variety. Data for this suite of 'functional markers' will be collected on a total of 184 varieties and the performance and potential uses of genotypic data in streamlining the DUS process will be examined.

3. We have identified and tested a cheaper alternative to the previously used Taqman system for the generation of genotype data. The system, provided as a service by KBiosciences (http://www.kbioscience.co.uk/), is based on their recently developed singleplex technology, KASPar, which dispenses with the need for the individually labeled fluorescent probes that are used in the Taqman system. This is the first high throughput genotyping platform on which a variable customized set of up to 96 SNP and insertion/deletion (InDel) variants could be assayed in parallel on multiple samples at low cost.

2. OBJECTIVES

1. Design and implement KASPar assays for a set of 96 trait-associated functional polymorphisms in barley.
2. Validate these assays in high throughput format and gather data on 184 barley varieties.
3. Use cluster analysis to compare similarity between varieties of common knowledge as measured by the selected eight DUS characteristics and by the markers developed.
4. Make recommendations on the potential to implement marker assays to streamline workload in existing DUS phenotyping.

3. BRIEF OVERVIEW OF APPROACH AND WORK PLAN

1. KASPar probe designs for 96 barley genotyping assays targeting functional polymorphisms in DUS characteristics and selected VCU traits.
2. Validation of the individual assays on control DNAs in ABI 7900 genotyper.
3. Validation of assay performance in high-throughput Fluidigm Biomark format on 96 parallel samples.
4. Estimation of diagnostic/predictive value of each group of allele-specific assays with corresponding phenotype.
5. Advice on the extent to which candidate varieties can be placed in the correct phenotypic grouping using only genotype data and the opportunities and limitations foreseeable for the application of a UPOV "Option 1(a)"[*]-type approach in barley.

4. RESULTS TO DATE

Objective 1

4. As part of another ongoing project, we have trialled the KASPar technology using a panel of 96 varieties of the complex diploid species *Vicia faba* L. (field bean). Of the 80 single nucleotide polymorphisms (SNPs) assayed, 75 resulted in robust assays. Our results using the KASPar platform demonstrate: (a) our in-house DNA extraction methods are of suitable quality for the generation of reliable genotype data; (b) the KASPar system is a suitable platform for efficient genotyping of large-genome plant species; and (c) efficient adaptation of SNP assays to the KASPar platform, and quick turn-around times. An example of the genotype data obtained is provided in **Figure 1**.

5. As the platform was found to be suitable for plant species with complex genomes, we proceeded with the design of the genotype assays for barley. For each gene, one or more polymorphic DNA features were assayed, with the following details recorded for each: (a) DNA sequence in FASTA format, with the polymorphism identified using standard nomenclature; (b) the GenBank accession number for the DNA sequence of the reference allele; (c) a PMID number, linking to the relevant scientific publication describing the allelic variants; (d) genetic map position of the gene assayed; (e) and information describing the SNPs and their associated phenotypes. Where we had sequence information in additional varieties, polymorphisms that had been identified in addition to the SNP to be assayed were also annotated on the sequence files, as their presence could affect the robustness of the assay.

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[*] Option 1: Molecular characteristics as a predictor of traditional characteristics
(a) Use of molecular characteristics which are directly linked to traditional characteristics (gene specific markers)

A total of 60 assays designed from 27 barley genes have been designed in the first instance, and the FASTA sequence files submitted to KBiosciences for assay design. We are expecting results from these by May 2010.

Objective 2

6. In order to validate the assays designed, an appropriate panel of barley varieties was collated that maximised the probability of encompassing all of the relevant genetic variants. This validation panel consists of 96 predominantly European varieties, selected to represent the most influential barley varieties grown in Western Europe over the last 50 years. The collection includes varieties belonging to different end-use categories (malting or animal feed) and to the major agronomic groupings (spring-/winter-sown and 2-/6-row ear types). The selection of germplasm that contains examples of each of the polymorphisms to be assayed for is essential for the marker development phase, as all genetic variants are not expected to be present in a United Kingdom-only panel. Once it has been determined that the assays designed are able to discriminate between the alternative alleles, they are then to be applied to the panel of 184 United Kingdom varieties. The varieties included in the validation panel are listed in **Table 1**. Seeds for the chosen varieties were sourced from national and international gene-banks, and plants grown to the 2-leaf stage. Leaf material from a single plant from each variety was used for genomic DNA extraction, using the DNAeasy 96 Plant Extraction Kit (Qiagen). DNA quality was assessed by running 2µl aliquots of each extraction on an ethidium bromide stained 1.5% agarose gel, and visualised under UV light. In addition, DNA quantity was determined using a Nanodrop 200 spectrophotometer (Thermo Scientific). All DNA extractions were found to be of sufficient quality and quantity, and were subsequently diluted to a final DNA concentration of 7ng/µl. The DNA samples for all 96 varieties in the validation panel have been sent to KBiosciences, and we are currently awaiting delivery of the resulting genotype data.

7. In addition, a panel of 184 United Kingdom barley varieties for genotypic analysis according to the project outline has been constructed, and seeds obtained. Genomic DNA has been extracted for the complete United Kingdom panel, and quality checked as described above.

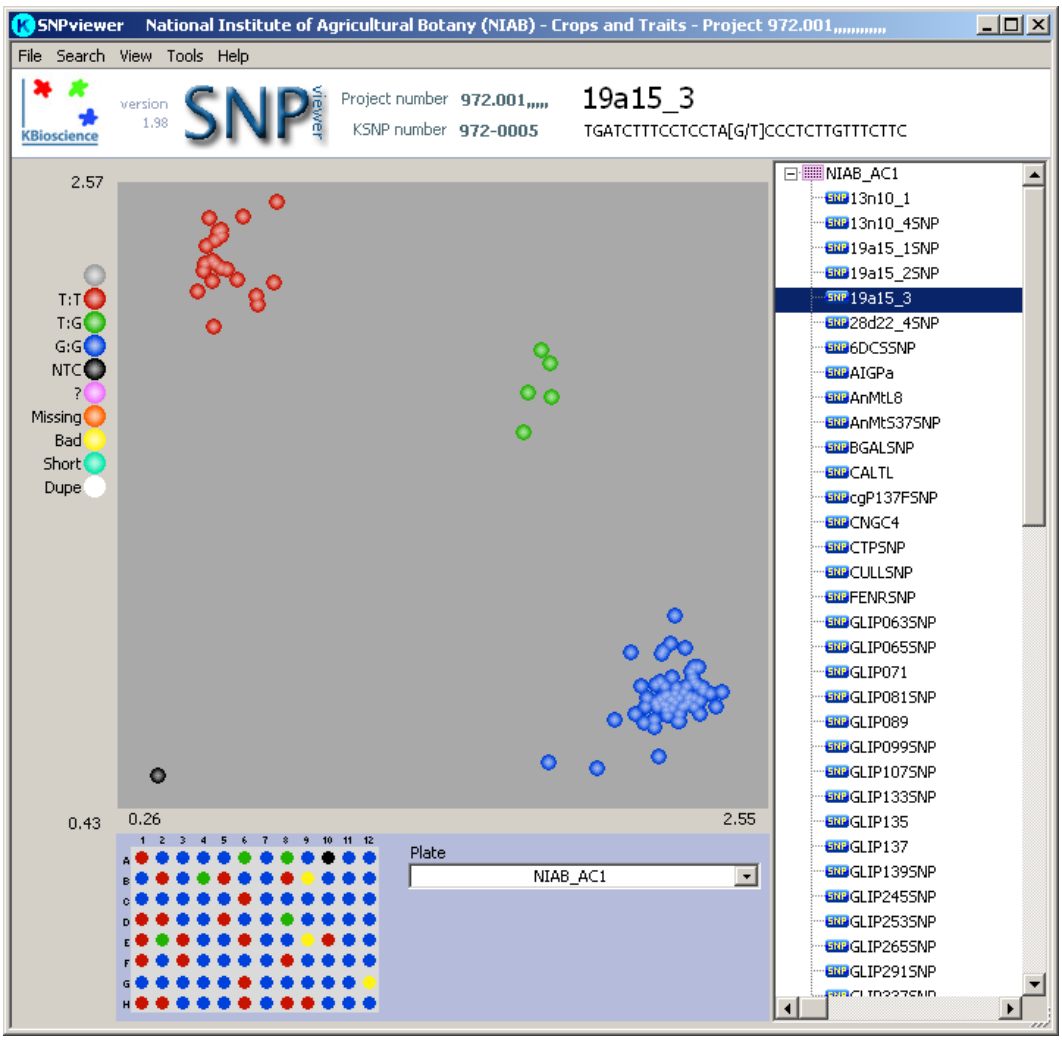

Figure 1. Visualization of SNP data generated from a single assay in *Vicia faba* using software freely distributed from KBiosciences. In this example, the alternative G/T SNP genotypes are clearly distinguishable (G = blue, T = red), with heterozygous individuals unambiguously clustered in a separate cloud (green).

Table 1. Barley varieties selected for the validation panel

Number	Variety	Seasonal growth habit	Ear row-number
1	Ager	Winter	6
2	Albacete	Winter	6
3	Alpha	Winter	2
4	Angora	Winter	2
5	Apex	Spring	2
6	Aramir	Spring	2
7	Armelle	Spring	2
8	Atem	Spring	2
9	Athos	Spring	2
10	B83-12/21/5	Spring	2
11	Barberousse	Winter	6
12	Baronesse	Spring	2
13	Beka	Spring	2
14	Betzes	Spring	2
15	Binder	Spring	2
16	Blenheim	Spring	2
17	Camargue	Spring	2
18	Carafe	Spring	2
19	Carlsberg	Spring	2
20	Carsten 2-row	Winter	2
21	Cebada capa	Spring	2
22	Chariot	Spring	2
23	Chime	Spring	2
24	Corniche	Spring	2
25	CPBT-B75	Spring	2
26	CPBT-B76	Spring	2
27	Cyrrhus	Winter	2
28	Derkado	Spring	2
29	Diamant	Spring	2
30	Dicktoo	Winter	6
31	Doyen	Spring	2
32	Dura	Winter	6
33	Emir	Spring	2
34	Fanfare	Winter	2
35	Fighter	Winter	2
36	Finesse	Winter	2
37	Franka	Winter	6
38	Friedrichsw. Berg	Winter	6
39	Ginso	Winter	6
40	Golden Promise	Spring	2
41	Gull	Spring	2
42	Haisa I	Spring	2
43	Halcyon	Winter	2
44	Harrington	Spring	2
45	Hatif de Grignon	Winter	6
46	Hauters (Nymphe)	Winter	6

47	Henni	Spring	2
48	Herfordia	Winter	6
49	Igri	Winter	2
50	Ingrid	Spring	2
51	Isaria	Spring	2
52	Kenia	Spring	2
53	Labea	Winter	2
54	Lina	Spring	2
55	Logan	Spring	2
56	Malta	Winter	2
57	Marinka	Winter	2
58	Maris Otter	Winter	2
59	Mehola	Winter	2
60	Meltan	Spring	2
61	Mokusekko 3 (M52)	Winter	6
62	Nudinka	Spring	2
63	Nure	Winter	2
64	Optic	Spring	2
65	Panda	Winter	2
66	Pastoral	Winter	2
67	Pearl	Winter	2
68	Pioneer	Winter	2
69	Pipkin	Winter	2
70	Plaisant	Winter	6
71	Prisma	Spring	2
72	Proctor	Spring	2
73	Puffin	Winter	2
74	Ragusa	Winter	6
75	Regina	Spring	2
76	Scarlett	Spring	2
77	Sergeant	Spring	2
78	Sonja	Winter	2
79	Spratt Archer	Spring	2
80	Tipple	Spring	2
81	Tocada	Spring	2
82	Torrent	Winter	2
83	Tremois	Spring	2
84	Tria	Winter	2
85	Triumph	Spring	2
86	Tschermaks	Winter	2
87	Vada	Spring	2
88	Vanessa	Winter	2
89	Villa	Spring	2
90	Vogels Gold	Winter	6
91	Volla	Spring	2
92	Waggon	Spring	2
93	Warboys	Winter	2
94	Westminster	Spring	2
95	Wisa	Spring	2
96	Zephyr	Spring	2

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