E

BMT/13/5
ORIGINAL: English
DATE: November 17, 2011

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

DEMONSTRATION OF SIGNIFICANT PROGRESS TOWARDS AN OPTION 1 APPROACH IN BARLEY

Document prepared by experts from the United Kingdom

SUMMARY

1. For the first time in a major United Kingdom arable crop species, it is possible to constitute a collection of DNA markers which assay for proven functional polymorphisms in genes underlying variation in Distinctness, Uniformity and Stability (DUS) characteristics. From an original panel of 82 markers, a validated set of 59 robust marker assays was assembled. These were either diagnostic for known functional alleles in DUS or Value for Cultivation and Use (VCU) traits, or tightly linked to (and therefore predictive of) phenotypic status. Data were collected on 169 United Kingdom varieties for this suite of 'functional markers'. The performance and potential uses of genotypic data in streamlining the DUS process were examined, indicating that there is potential to use the suite of markers either to identify the closest varieties at an earlier stage than is possible in the field, or to avoid growing some reference varieties for comparison with candidates. This could reduce the number of varieties that need to be grown in the field.

BACKGROUND AND PURPOSE

2. To date, the use of molecular markers as a predictor of traditional characteristics ("Characteristic-specific molecular markers": previously known as "Option 1"*) has received relatively little attention. Previously, it was shown that limited validation work following the

[*]original identification of genes underlying a DUS characteristic allowed the design of diagnostic genetic markers predictive of 'spring' or 'winter' seasonal growth habit in barley (Final Report VS0137; Cockram *et al* 2007a, 2007b, 2009). The development of molecular markers for this trait was taken forward in a subsequent project (2007J "Functional SNP Markers for the Vernalization Requirement in Barley"), designed to address the difficulty this system had in predicting those varieties with little or no vernalization requirement (classified as 'alternative' varieties). The molecular tests developed can be performed within days of receipt of a candidate variety, and have the potential to negate the need for a dedicated field trial to assess seasonal growth habit.

3. The recent cloning of the row-number genes *Vrs1* ('Ear: number of rows', CPVO number 11G, Komatsuda *et al* 2007), *Cly1* ('Grain: disposition of lodicules' CPVO number 26, Nair *et al* 2010) and *int-C* ('Ear: number of rows', CPVO number 11G, Waugh *et al* unpublished) highlight the increasing relevance molecular markers could have in relation to DUS testing in barley.

4. As part of the BBSRC LINK project 'Association Genetics of UK Elite Barley' (AGOUEB) in which all barley varieties which were National Listed from 1993 to 2005 were genotyped at 1,536 single nucleotide polymorphism (SNP) loci, genome-wide association (GWA) mapping was used to detect associations between molecular markers and DUS characteristics for which sufficient phenotypic data was available. Of the twenty-eight characteristics currently used in DUS testing, significant loci controlling ten additional traits were genetically mapped to a resolution of ~5 cM: 'Lower leaves: hairiness of leaf sheath' (CPVO-TP 019/2 rev number 2), 'Flag leaf: intensity of anthocyanin coloration of auricles' (3), 'Awns: intensity of anthocyanin coloration of tips' (7), 'Ear: development of sterile spikelets' (18), 'Sterile spikelet: attitude (in mid third of ear)' (19), Grain: rachilla hair type' (21), 'Grain: anthocyanin coloration of nerves of lemma' (23), 'Grain: spiculation of inner lateral nerves of dorsal side of lemma' (24), 'Grain: hairiness of ventral furrow' (25G) and 'Kernel: colour of aleurone layer' (27). Candidate genes in the immediate vicinity of these association peaks were also assessed for 'causative' polymorphisms rather than 'linked' SNPs.

5. Developments in the genetic understanding of DUS characteristics are now being matched by advances in relatively cheap genotyping platforms that can be flexibly deployed to assay small sets of user-defined markers. Therefore, the opportunity exists to assess the currently available suite of linked and putatively causative SNPs for their ability to predict individual characteristic states. To maximize the potential informativeness of the assays undertaken, some functional polymorphisms which tag alleles of cloned barley resistance genes and VCU-type quality traits were also included in the study.

Objectives

1. Design and implement Taqman 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.

---

[*] Referred to as 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)" in document TC/38/14-CAJ/45/5

3. Use cluster analysis to compare similarity between varieties in 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 the current DUS testing system.

RESULTS

OBJECTIVE 1

(a) *Identification of relevant genetic loci*

6. Initially, a survey of DUS characteristics was carried out for which genetic loci or the underlying genes involved had previously been identified. Literature searches identified mapped genetic loci corresponding to 12 of the 28 DUS characteristics. A list of the 28 characteristics currently scored under the DUS system, alongside relevant genetic loci, is presented in Table 1.

7. As well as genetic loci and genes relevant to DUS characteristics, a survey of cloned genes controlling disease resistance and VCU related quality traits (Table 2) was also included. Seven relevant traits were selected, represented by six cloned genes (the powdery mildew genes *Mla* and *Mlo,* stem rust resistance genes *Rpg1* and *Rpg5* and the yellow mosaic virus resistance gene *Rym4/5*) as well as one fine-mapped locus (the leaf scald resistance locus, *Rrs2*) and three candidate genes (grain hardness candidate gene *Hin-b,* malting quality gene *Bmy1* and crude protein extract candidate, *HvBPBF*).

(b) *Identification of relevant genetic markers*

8. Once the genes to be assayed had been identified, selected genetic polymorphisms were chosen for subsequent genotyping. In an effort to further reduce operating costs, a cheaper alternative to the Taqman system was tested for the generation of genotype data. The KASPar platform, provided as a service by KBiosciences (http://www.kbioscience.co.uk/), is based on their single-plex technology which dispenses with the need for the individually labelled fluorescent probes used in the Taqman system. Previous work carried out at NIAB on field bean (*Vicia faba*) has shown KASPar to be suitable for plant species with complex genomes such as barley and was therefore used for the design of genotype assays for barley. For each selected gene, one or more polymorphic DNA features were assayed, with the following minimum details recorded for each: (a) DNA sequence, with the targeted polymorphism identified using standard nomenclature (eg presented in Figure 1) (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) Information describing the SNPs and their associated phenotypes. Where previously generated sequence information in United Kingdom germplasm exists, polymorphisms that had been identified in addition to the SNP to be assayed were annotated within the sequence files, as their presence could affect the robustness of the assay.

9. A total of 86 assays from 51 barley genes were submitted to KBiosciences for conversion, returning 82 putatively possible assays (putative conversion rate = 95 %). Conversion failure was due to lack of appropriate sites within the sequence for primer design.

OBJECTIVE 2

(a) *Genetic marker validation*

10. A panel of 90 barley varieties was selected in order to validate the KASPar assay designs (Annex 1). This panel was selected to represent a range of varieties of predominantly north-western European origin, many of which have been prominent in the pedigrees of modern United Kingdom varieties. 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), with the aim of ensuring a good representation of the likely allelic variants found in the current elite United Kingdom gene pool. Seeds for the chosen varieties were sourced from national and international gene-banks, and plants grown to the two leaf stage. Leaf material from a single plant for each variety was used for genomic DNA extraction, using DNAeasy 96 Plant Extraction Kits (Qiagen). DNA quality was assessed by running 2 µl 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 7 ng/µl with sterile water. The DNA samples for all 90 varieties in the validation panel were sent to KBiosciences for genotyping. Each assay required 1 µl of genomic DNA as template. A single well was reserved as a negative water control.

11. Genotyping results were returned as .csv files, and viewed using the freeware, SNP Viewer v1.99 (https://kbiosciences.co.uk). Of the 82 assays that were converted to the KBiosciences platform, three were found not to work when applied in practice. The remaining 77 assays are listed in Table 3 (supplied as an Excel file). A further 20 assays were found to be unreliable (highlighted in grey in Table 3) due to: (1) insufficient separation between allele clusters, or (2) inability of the KASPar platform to appropriately convert assays that test for Insertion/Deletion (InDel) genetic polymorphisms. Although the latter observation is problematic if a unified platform for diagnostic markers in barley is required, in practice it should be easily overcome, as the nature of InDel polymorphisms makes them simple and cheap to genotype by electrophoretic separation of PCR amplification products across agarose gels (eg *ANT2*: Cockram *et al* 2010; *VRN-H1*: Cockram *et al* 2009; ZCCT-*Ha, -Hb, -Hc,* Karsai *et al* 2005). Insufficient allelic discrimination appeared to be prominent in genes or loci which may represent high copy number loci (eg disease resistance genes) within the barley genome, a problem common to all high-throughput genotyping platforms. Of the 57 assays that were considered reliable from the validation plate, the mean missing score rate was ~1 %, indicating a high genotyping success rate within this set. Proportions of unknown calls for each marker, as well as SNP allele frequencies are listed in Table 3.

(b) *Genotyping validated markers in UK germplasm*

12. Of the final collection of 57 assays, markers 1 – 37 are relevant to DUS characteristics, while markers 38 – 57 represent genes related to VCU/quality traits. A panel of 169 United Kingdom barley varieties was collated for genotypic analysis with the validated marker set (Appendix 2). Genomic DNA was extracted and quality checked as described previously. Genotyping of the United Kingdom varieties with the 57 validated KASPar markers set returned ~10,000 high quality data-points (Appendix 2), with a mean missing score rate of <1 %. Allelic frequencies are summarised in Table 3. A wide range of minor allele frequencies (MAF) were observed, ranging from 0.01 to 0.49 (mean MAF = 0.23, median MAF = 0.24). Three DUS related markers were found not to be polymorphic in the United Kingdom set: marker HvBRI1_A2570G represents a putative diagnostic SNP at the *uzu* dwarfing gene.

This polymorphism is widespread in Asian barley and somewhat unsurprisingly appears not to be deployed in United Kingdom germplasm. While marker HvSdw1_AG tags a polymorphism within the candidate gene for the *Sdw1* locus controlling height, it was identified within a bi-parental mapping population and does not necessarily represent the causative genetic variant. Lastly, although HvCly1_A2664C represents a causative mutation for openness of flowering (a trait associated with lodicule disposition), open-flowering varieties with the 'frontal' bib disposition (CPVO characteristic 26) are very rare in modern United Kingdom varieties. Two of the twenty VCU/quality related markers were found to be monomorphic: one marker from the grain hardness candidate gene *Hin-b* (Hinb_C262T) and one from *Bmy1* (HvBMY1_T698C). Subsequent analysis within this report focuses on DUS-related phenotypes/markers only.

OBJECTIVE 3

(a) *Interpretation of DUS marker genotypes*

13. Fifty percent of the 28 characteristics currently scored under DUS were represented by molecular markers, originating either from relevant candidate genes or from flanking makers. In order to determine the predictive value of genetic markers, we constructed a phenotypic database for the current set of 28 DUS characteristics, scored across the 169 United Kingdom varieties in this study (Table 4). Each trait differed in the number of records that were available, ranging from 76 % fill (CPVO character 19) to 95 % (CPVO characters 11G, 21 and 25G). The predictive values (based on the percentage of correctly called trait scores, as predicted by marker genotype) of genetic markers for their relevant characteristic varied widely (summarised in Table 5**)**. The highest values were obtained from markers originating from cloned genes, demonstrating the utility of continued investigation of the molecular mechanisms controlling phenotypic variation. For example, the genes underlying genetic control of 'Seasonal growth habit' are now relatively well characterised. However, due to the number of different alleles at the *VRN-H1* locus that control spring seasonal growth type, it is difficult to accurately predict this phenotype using a single genetic marker. In addition, the functional polymophisms involved are large InDels, which are problematic for the KASPar platform to assay. Nevertheless, a combination of two KASPar markers (HvVRNH1_SNP2 and HvVRNH1_hap2_InDel), have a predictive value of 92%. This marker combination is unable to distinguish the spring *VRN-H1* haplotype 1B from winter haplotype 1A (see Cockram *et al* 2007 for a full description of *VRN-H1* haplotypes), providing an explanation for the 8% of incorrectly predicted trait scores. A predictive power of 100% is achieved using the PCR-based *VRN-H1* marker (Cockram *et al* 2009) previously developed as part of project VS0137.

14. For the characteristic 'ear: number of rows', marker Hv11_20606_GC was found to perfectly predict the 2- or 6-row phenotype. This is an interesting result, as this marker maps to a different chromosome (4H) to the gene that is known to control this trait (*VRS1* on chromosome 2H). The presence of a predictive marker unlinked to *VRS1* is because Hv11_20606_GC is predicted to map close to the ear morphology locus *Int-C*, which modifies the degree of fertility of lateral spikelets (Komatsuda *et al* 2002; Waugh *et al* 2009). Perfect allelic partitioning between 2 and 6-rowed barley for *int-c* alleles that either prevent or promote anther development in lateral spikelets explains the perfect association with ear row-number observed. Of the three mutations at *VRS1* that result in 6-row alleles, only two were found to be convertible to the KASPar platform (HvVRS1_C349G and HvVRS1_GINS681). Therefore, the haplotypes derived from the assayed *VRS1* markers displayed a slightly lower predictive value of 95%.

15. A marker prediction score of 100% was observed for 'Grain: disposition of lodicules' (CPVO number 26) marker HvCLY1_A2604G, which assays for one of the two mutations at the *CLY1* gene *HvAP2* previously shown to control this characteristics (Nair *et al* 2010). Only one variety within the United Kingdom panel was recorded as possessing the frontal 'bib' type lodicules disposition (AFP 2/1091), which was predicted by the presence of SNP G at HvCLY1_A2604G. Although the second mutation at *CLY1* was also assayed (HvCLY1_A2662C), this marker was found to be monomorphic in the UK varietal panel.

16. Allelic state at the marker Hv11_11299_GC was found to have a 96% predictive value for the characteristic 'Lower leaves: hairiness of leaf sheaths (CPVO number 2G), with SNP A and G predictive of the absence (score 1) and presence (score 9) of hairs, respectively. This SNP is currently the closest genetic marker to the *HSH1* locus that is known to control the phenotype. This characteristic presents itself as a good candidate for map-based cloning, as it represents a simply inherited DUS characteristic which is physically onerous to score in the field.

17. Quantitative characteristics are more problematic for development of molecular markers as diagnostic tools, as the levels of underlying molecular control is much more complex than for qualitative characteristics. However, we have previously shown that fitting binary genetic markers to such characteristics in some cases does result in the identification of markers significantly associated with the characteristic (Cockram *et al* 2010). In practice, this often means that one allele is associated with a single characteristic score (eg absence), while the alternative SNP is associated with the remaining characteristic scores (eg various degrees of presence). This is true of four DUS characteristics for which good marker predictions (>85%) were obtained: CPVO characteristics 3, 7, 19, 23 and 27.

18. In the case of the anthocyanin intensity related characteristics (3, 7 and 23), a master switch was identified for the ability of the plant to synthesise anthocyanin (Cockram *et al* 2010): an InDel within exon 6 of the underlying gene (*HvbHLH1*) results in a severe truncation of the predicted protein, resulting in the inability to synthesise anthocyanin. As the KASPar platform was unable to assay this InDel directly, a SNP marker was designed from a closely linked polymorphism within the gene (HvANT2_C4289T). For these three related anthocyanin related characteristics, SNP T is predictive of score 1 (absence of anthocyanin), while SNP C is predictive of scores 2-9 (increasing intensity), returning predictive values for CPVO characteristics 3, 7 and 23 of 98% 97% and 92%, respectively. As the assayed marker is not the causative polymorphism, it is possible that these predictions may improve with the deployment of the PCR/agarose gel-based marker for the *HvbHLH1* InDel, or by further efforts to convert the assay to the KASPar platform.

19. For characteristic 'Sterile spikelet: attitude' (UPOV character 19), marker Hv11_10933_GC SNP G is predictive of scores 1 (parallel) and 2 (parallel – divergent) while SNP C is predictive of score 3 (divergent), resulting in a predictive value of 88%. This marker is located on chromosome 1H, and is predicted to represent natural variation at the induced mutant locus *VRS3*, which controls related lateral spikelet morphology. Its location within the centromeric region suggests that although fine-mapping and cloning of the gene may be problematic, the reduction in recombination in the region also means that flanking markers may well prove to be in very strong LD (and therefore highly diagnostic) with *VRS3*.

20. CPVO characteristic 27 ('Kernel: color of aleurone layer') is a 3 state characteristic, for which marker HvOs03g14250_C82T displays a predictive value of 92%. This is based on

SNP G predicting score 1 (white) and SNP A predictive for scores 2-3 (weakly – strongly coloured). The gene determining this characteristic is currently subject to fine mapping as part of further work.

(b) *Comparison of cluster analysis based on phenotypes and molecular markers.*

21. To determine the effectiveness of varietal discrimination using genetic markers *versus* their corresponding phenotypic assessments, we performed cluster analysis based on the twelve DUS characteristics for which genetic markers proved highly predictive, and their twelve corresponding genetic markers: CPVO traits 1 (HvFT3), 2G (HvOs03g01380_A447G), 3 (HvANT2_C4289T), 7 (HvANT2_C4289T), 11G (Hv11_20606_GC), 19 (Hv11_10933_GC), 21 (Hv11_20850_AG), 23 (HvANT2_C4289T), 25G (HvOs02g01490_G607A), 26 (HvCLY1_A2604G), 27 (HvOs03g14380_G125A) and 28G (HvVRNH1_SNP2_0P5_HAP, which represents a combined haplotype from two of the KASPar assays). All varieties with < 50% missing data in either set (phenotypic or genotypic) were removed, leaving 158 varieties for subsequent analyses. Inter variety distances were calculated using Rogers distance for the genotypic data (using Powermarker v2.35) or Euclidean distance for the phenotypic data (R statistics package). These matrices were used to calculate Neighbor-joining trees based on phenotypic (Figure 3) and genotypic (Figure 4) data. The two distance matrices produced were compared by calculating a correlation coefficient. The correlation was both high and positive (0.72) and shown to be highly significant ($p<0.001$) by permutation. To visualize more effectively the congruence between the genotypic and phenotypic clustering, the respective distance trees shown in Figures 3 and 4 are reproduced side-by-side on a smaller scale in Figure 5 with colored lines joining corresponding AFP codes. Cluster analysis using the twelve phenotypes was able to uniquely identify 88% of the varieties included. However, we note that cluster analysis using the corresponding molecular markers was not able to achieve comparable resolution, with two large groups of 22 and 34 spring 2-rowed varieties showing 100% genotypic identity. Interestingly, the genotypic cluster analysis appeared to differentiate winter varieties more readily than spring varieties, with the largest undifferentiated winter cluster consisting of six varieties. This is consistent with spring barley varieties being more difficult to distinguish from each other by phenotype than winter barley varieties. It was also due at least in part to the following two reasons: (1) multi-character scores for a single phenotypic trait provide greater potential for differentiation compared to the equivalent bi-allelic genetic marker (2) a single bi-allelic genetic marker was used to predict phenotype in three different DUS characteristics, giving less differentiation than the phenotypic scoring. Despite the shortcomings listed above, this study demonstrates the potential that deployment of larger numbers of genetic markers may have in varietal discrimination.

OBJECTIVE 4

(a) *Recognition of characteristics for which markers could be readily implemented within the DUS system*

22. While cluster analysis showed that the use of genetic markers in place of phenotypic assessment for the unique identification of varieties is likely to be possible, a major barrier to the deployment of genetic markers for the prediction of DUS phenotypic scores is that while the former are essentially binary scores, the latter are often scored using three or more characteristic states. For this quantitive characteristics, it is unlikely molecular markers will soon be able to provide a like-for-like replacement of phenotypic scores. Nevertheless, within

the collection of markers assayed, a range of predictive power was observed. These can be divided into three broad categories:

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

23. Molecular markers for three traits 'Ear: number of rows', 'Grain: disposition of lodicules' and 'Seasonal growth habit' were found to be 100% predictive of phenotypic state. We note that although the marker assaying for one of the two causative polymorphisms controlling 'Grain: disposition of lodicules' was found to be diagnostic in our sample, it was not possible to design a KASPar assay for the second causative SNP. As alleles at the second SNP are rare, the absence of these assays did not lower the predictive value of the assays. However, to be truly diagnostic in all instances, it would be necessary to attempt alternative primer designs in order to be able to assay for both causative polymorphisms.

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

24. This group is predicted to contain characteristics with binary or three-state phenotypic scores, for which the gene/genetic variant underlying Mendelian genetic locus has yet to be cloned. This is true of the characteristics 'Kernel: color of aleurone layer' and 'Lower leaves: hairiness of leaf sheaths', whose high (but not perfect) predictive power is likely due to the fact that the genetic markers assayed originate from a closely linked loci, and not from causative polymorphism within the underlying genes. Work is underway for both of these traits, and diagnostic markers for both should soon be achievable. A molecular marker for the three anthocyanin intensity characteristics (CPVO numbers 3, 7 and 23) are highly predictive for the presence/absence of anthocyanin, but are unable to determine intensity when present.

Group 3: Characteristics which provide good (>80%) phenotypic prediction by markers

25. This class of characteristics are similar to group 2, but lag behind in the genetic characterization and mapping of the loci involved. They are quantitative characteristics with lower genetic heritabilities. Although improvement in marker-characteristic correlations could be predicted within this class through even better marker coverage and more comprehensive definition of QTL underlying quantitative aspects of phenotype, progress could be expected to be slow and incremental.

(b) *Choice of marker platform for implementation*

26. At present, with a limited number of assays (13) which give perfect or very good predictions of actual DUS phenotype, we believe that the KASPar assays described here supplemented by some agarose-based INDEL analysis (as for the *VRN-H1* indels) represents the most economical way to capture low-throughput genotype data.

27. The full economic cost of extracting a bulked barley sample for DUS testing and applying the 13 DUS-predictive SNPs described is estimated at approx. £3.60 per variety (£1.50 for DNA extraction and £2.10 for the SNP analysis). Given the low cost of obtaining this first-pass genotypic description of the candidate variety, the assay could potentially be used for the following purposes:

1. To keep the molecular database updated. 2. To help group similar varieties in the field e.g. keeping varieties with absent anthocyanin to one side of the field could help get a better comparison of degree of anthocyanin expression in the remainder. However, this test

currently lacks discriminatory power and only the case of two characteristics – hairiness of leaf sheaths and absence of anythocyanin pigmentation of auricles, awns and lemma nerves – does the genotype data add immediately to what can be ascertained by careful examination of a grain sample, which means that major changes to the overall DUS testing regime are not yet recommended.

(c) *Perspectives:*

28. As noted in this report, work is ongoing towards the identification of causative polymorphisms underlying DUS characteristics including aleurone color and hairiness of leaf sheaths. As genes continue to be identified for DUS characteristics which are difficult to assess phenotypically, we envisage a point where the rationale for molecular profiling of candidate varieties grows and will allow rationalization both in the total number of varieties tested and the extent of manual phenotyping. For example, the normal selection of control varieties which attempt to capture the extremes of every phenotypic state, could be replaced by the set of lines predicted to be most similar to the candidate varieties. In another instance, it may be possible in the future to record a particular phenotype as "read by genotype" e.g. hairiness of lower leaf sheath, saving days of field work at a particular growth stage, even though the trial itself and the collection of some categories of phenotypic data cannot be avoided.

CONCLUSIONS

29. Results reported here are based on the analysis of one plant and do not take into account uniformity, an important consideration of the DUS process. Further work would be needed in this area before any of these assays could be used directly to replace the field test. To do this, dilution series would be set up with contrasting pairs to sample all the alleles, followed by bulk extractions to detect off types. This approach proved to be successful in the project 2007J "Functional SNP Markers for the Vernalization Requirement in Barley".

30. The results presented here are very promising for the future use of molecular markers within the barley DUS testing system and represent significant progress in the characterisation of barley varieties by genotyping. The results show that the phenotypic measurement of some DUS characteristics (those with 100% phenotypic prediction falling into the Group 1 category) could potentially be replaced by molecular marker assays in an Option 1-type model if economically viable and reliable for uniformity assessment.

31. The characteristics falling into this category are qualitative characteristics such as 'Ear: number of rows', 'Grain: disposition of lodicules' and 'Seasonal growth habit', however 'Ear: number of rows' is a quick and easy assessment in the field and although 'Grain: disposition of lodicules' can be a difficult phenotypic assessment to make, only one variety within the United Kingdom panel was recorded as possessing the frontal 'bib' type lodicules disposition. An assay for 'seasonal growth type' is already available from work carried out in project 2007J "Functional SNP Markers for the Vernalization Requirement in Barley" and is currently being considered for implementation following submission of the final report and presentation of the results at the last UPOV BMT meeting in May 2010. Replacing field assessment with assays from the Group 1 category would not currently return any efficiency savings when used in isolation.

32. For those characteristics which fall into the Group 2 category; characteristics which provide very good (>90%phenotypic prediction), it is a possibility that the predictive power of the molecular assay could be as good as, if not better than the accuracy of the phenotypic assessment. Phenotypic assessment can never be 100% accurate as it relies on a certain degree of subjectivity and the influence of environmental factors, in spite of every effort to limit these influences. Therefore a predictive power of more than 90% ought to be sufficient when combined with other characteristics to enable these assays to be used confidently. The characteristics 'Kernel: color of aleurone layer' and 'Lower leaves: hairiness of leaf sheaths' can be time consuming when assessed phenotypically and would benefit from replacement with a molecular assay, however further work is needed and is in progress to improve the assays by using the causative polymorphism within the underlying genes rather than genetic markers assayed originating from closely linked loci.

33. Assays for characteristics falling into the Group 3 category are less likely to be used alone as they do not confidently predict the characteristic states, however they give an indication of the extreme ends of the character ranges. These assays could be used in combination with other assays from Groups 1 and 2 to give additional weight to differences between varieties, once the further developments have been made.

34. Although the results from this project are extremely promising, none of the assays developed can currently be used in isolation as they are either not cost effective to be used singly compared to the phenotypic assessment, or are not accurate enough to predict all states of a characteristic. Currently all of the ear assessments are carried out at the same time, so a replacement molecular assay for all or the most time consuming of those characteristics would need to be developed for it to be worthwhile. We would however envisage that further developments within Group 2 and 3 characteristics will allow a suite of markers for all characteristics assessed within this project to be used collectively in a single test to replace the phenotypic assessments. Alternatively new candidates could be assayed on receipt of the seed sample and compared to a database of molecular profiles of reference varieties. If the turnaround of sample receipt and molecular testing were sufficiently quick, this would enable reference varieties that were shown to be clearly distinct (a distinctness threshold would need to be established) from candidates to be excluded from the growing trial, thereby reducing the number of varieties that would need to be directly assessed in the field.

35. Given more accurate predictive powers of the Group 2 and 3 characteristics and further work on the possibility of detecting off-types with the KASPAR system several options will become available for use within the barley DUS testing system in the near future.

REFERENCES

Cockram J, Chiapparino E, Taylor SA, Stamati K, Donini P, *et al*. (2007a) Haplotype analysis of vernalization loci in European barley germplasm reveals novel VRN-H1 alleles and a predominant winter VRN-H1/VRN-H2 multi-locus haplotype. *TheorAppl Genet*115: 993-1001.

Cockram J, Mackay IJ, O'Sullivan DM (2007b) The role of double-stranded break repair in the creation of phenotypic diversity at cereal VRN1 loci. *Genetics* 177: 1-5.

Cockram J, Norris C, O'Sullivan DM (2009) PCR-based markers diagnostic for spring and winter seasonal growth habit in barley. *Crop Sci* 49: 403-410.

Cockram J, White J, Zuluaga DL, Smith D, Comadran J, *et al*. (2010) Genome-wide association mapping to candidate polymorphism resolution in the un-sequenced barley genome. Submitted.

Karsai I, Szűcs P, Meszaros K, Filichkina T, Hayes PM *et al*. (2005) The *Vrn-H2* locus is a major determinant of flowering time in a facultative × winter growth habit barley (*Hordeum vulgare* L.) mapping population. *Theor Appl Genet* 110: 1458–1466.

Komatsuda T, Yano Y (2002) Molecular mapping of the intermedium spike-c (*int-c*) and non-brittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.). *Theor Appl Genet* 105: 85-90.

Komatsuda T, Pourkheirandish M, He C, Azhaguvel P, Kanamori H, *et al.* (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. *Proc Natl Acad Sci USA* 104: 1424–1429.

Nair SK, Wang N, Turuspekov Y, Pourkheirandish M, Sinsuwongwat S *et al*. (2010) Cleistogamous flowering in barley arises from the suppression of microRNA-guided *HvAP2* mRNA cleavage. *Proc Natl Acad Sci USA* 107: 490-495.

Waugh R, Jannink J-L, Muehlbauer GJ, Ramsay L (2009) The emergence of whole genome association scans in barley. *Curr Opin Plant Biol* 12: 218–222.

Figure 1. Exemplar of DNA sequence format for each KASPar assay. The two allelic states at the target SNP are separated by the symbol '/' and enclosed within square brackets, and bases to avoid during primer design are replaced with the letter 'N'. Known polymorphic base-pairs within the flanking sequence are indicated using standard nomenclature: M = A/C, R = A/G, W = A/T, S = C/G, Y = C/T, K = G/T.

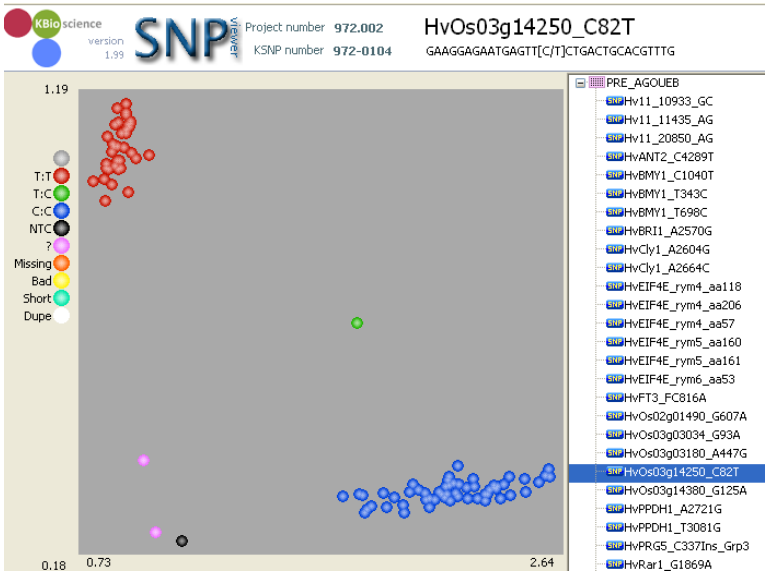

Figure 2. Visualisation of SNP data generated from assay HvOs03g14250_C82T, using SNP Viewer (KBiosciences). The alternative SNP genotypes are clearly distinguishable (T:T = red, C:C = blue), with heterozygous individuals (T:C = green) unambiguously clustered in a separate cloud. The water negative control is shown in black, while unknown calls (predicted to represent wells which lack DNA) are shown in pink.

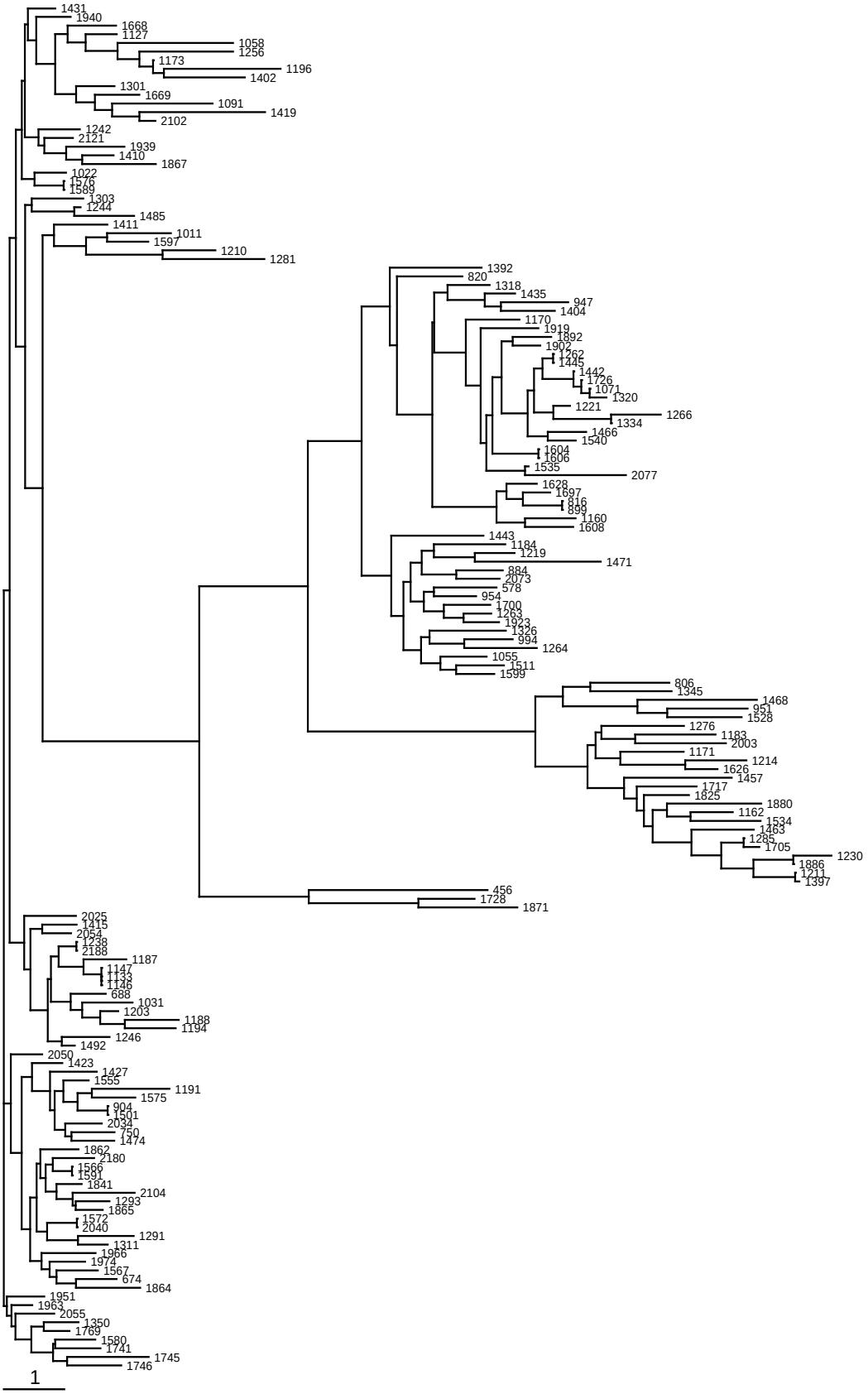

Figure 3. Euclidian distance tree of United Kingdom germplasm, based on phenotype.

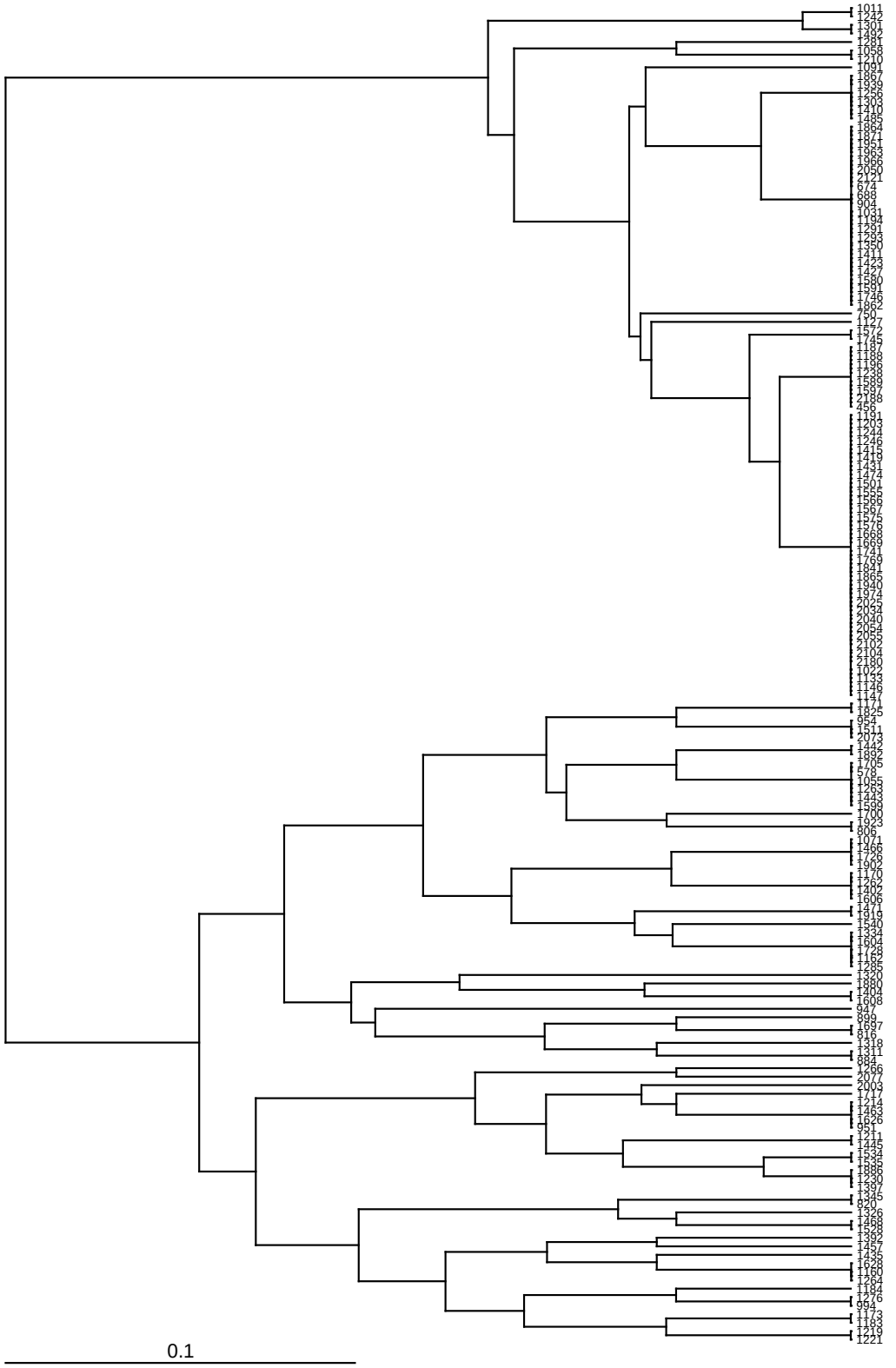

Figure 4 Rogers distance tree of United Kingdom germplasm, based on genotype.

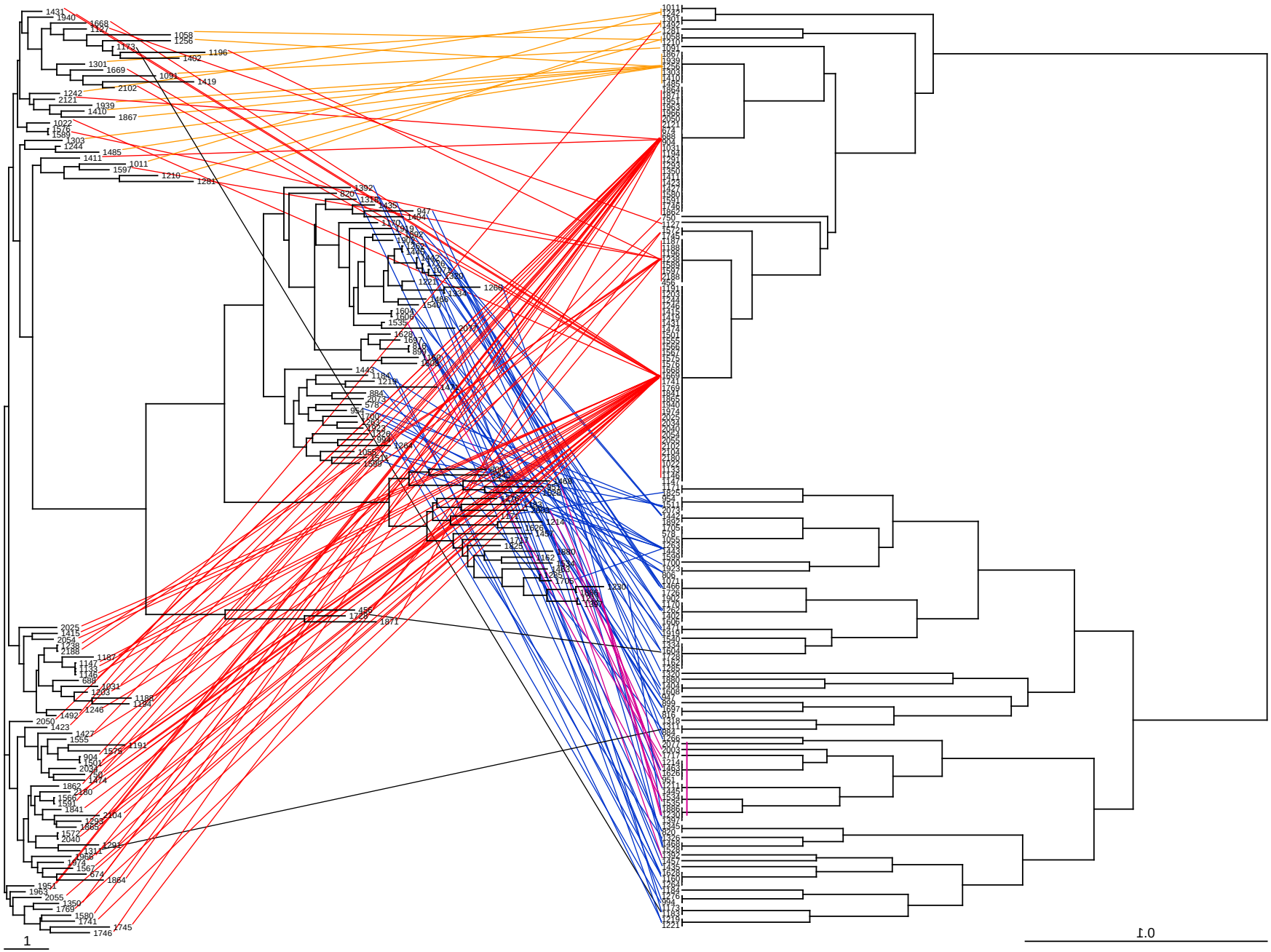
1
0.1

Figure 5: Variety-by-variety correspondences between phenotypic (DUS) and (Option I) genotypic clustering methods. Blue (2-row) and purple (6-row) lines join winter varieties and red and orange lines join spring two-row varieties.

CPVO No.	Characteristic	Known genetic loci (Chr)	GWAS loci (No.)	Progress towards cloning underlying genes
1	Plant growth habit	N/A	Y (1)	N/A
2G	Lower leaves: hairiness of leaf sheaths	*HSH1* (4H)	Y (1)	fine-mapping (at NIAB)
3	Flag leaf: intensity of anthocyanin colouration of auricles	*ANT2* (2H)	Y (2)	candidate gene
4	Plant: frequency of plants with re-curved leaves	N/A	N	N/A
5	Flag leaf: glaucosity of sheath	*Ecf* loci	N	N/A
6	Time of ear emergence (1st spike vis on 50% ears)	*PPD-H1* (2H) *PPD-H2* (1H)	N	candidate genes
7	Awns: intensity of anthocyanin colouration of awn tips	*ANT2* (2H)	Y (2)	candidate gene
8	Ear: glaucosity	*Ecf* loci	N	N/A
9	Ear: attitude (at least 21 days after ear emergence)	*VRN-H1* (5H)	Y	candidate gene
10	Plant: length (stem, ears and awns)	*HvBRI, Sdw1*	N	Candidate gene
11G	Ear: number of rows	*VRS1* (2H) *Int-c* (4H)	Y (2)	cloned gene (VRS1)
12	Ear: shape	N/A	N	N/A
13	Ear: density	N/A	N	N/A
14	ear length (excluding awns)	N/A	N	N/A
15	Awn length (compared to ear)	N/A	N	N/A
16	Rachis: length of first segment	N/A	N	N/A
17	Rachis: curvature of first segment	N/A	N	N/A
18	Ear: development of sterile spikelets	*VRS3* (1H) *VRS1* (2H)	Y (2)	cloned gene (VRS1)
19	Sterile spikelet: attitude (mid 1/3 of ear)	*VRS3* (1H) *VRS1* (2H)	Y (2)	cloned gene (VRS1)
20	Median spikelet: length of glume+awn+ cf grain)	N/A	N	N/A
21	Grain: rachilla hair type	*SRH*	Y (1)	fine-mapping (at SCRI)
22	Grain: husk	N/A	N	N/A
23	Grain: anthocyanin colouration of lemma nerves	*ANT2* (2H)	Y (2)	candidate gene
24	Grain: spiculation of inner lateral nerves	N/A	Y (1)	N/A
25G	Grain: ventral furrow - presence of hairs	N/A	Y (1)	
26	Grain: disposition of lodicules	*CLY1*	Y (1)	cloned gene
27	Kernel: colour of aleurone layer	*BLX1*	Y (1)	fine-mapping (at SCRI)
28G	Seasonal type	*VRN-H1* (5H) *VRN-H2* (4H)	Y (2)	cloned genes (*VRN-H1* and *VRN-H2*)

Table 1. Current progress towards understanding the genetic determinants controlling DUS traits. GWAS: genome-wide association scan. N/A: not applicable.

Characteristic	**genetic locus/gene**	**progress**
Powdery mildew resistance	*Mla*	Cloned
Powdery mildew resistance	*Mlo*	cloned
Stem rust resistance	*Rpg1*	cloned
Stem rust resistance	*Rpg5*	cloned
Leaf scald resistance	*RRS2*	fine-mapped
Yellow mozaic virus resistance	*Rym4/5*	cloned
Grain hardness	*Hin-b*	candidate gene
Malting quality	*Bmy1*	cnadidate gene
Crude protein extract	*HvBPBF*	candidate gene

Table 2. Barley VCU/quality traits for which genetic loci/cloned genes have been identified.

Table 3 (see Excel file). Details of the 77 KASPar assays designed for validation. Details include chromosome designation, reference DNA sequence (GenBank of HarvEST accession), allelic frequencies and reference to relevant articles (PMID number).

	AFP number \ Assay	PLANT - GROWTH HABIT	LOWER LEAVES - HAIRINESS OF LEAF SHEATHS	FLAG LEAF - INTENSITY OF ANTH. COLOUR. OF AURICLES	PLANT - FREQUENCY OF PLANTS WITH RECURVED LEAVES	FLAG LEAF - GLAUCOSITY OF SHEATH	TIME OF EAR EMERGENCE (1st spk. vis. on 50% ears)	AWNS-INTENSITY OF ANTHOCYANIN COLOUR. OF AWN TIPS	EAR - GLAUCOSITY	EAR - ATTITUDE (at least 21 days after ear emerg.)	PLANT - LENGTH(STEM,EARS AND AWNS)	EAR - NUMBER OF ROWS	EAR - SHAPE	EAR - DENSITY	EAR - LENGTH(EXCLUDING AWNS)	AWN - LENGTH (compared to ear)	RACHIS - LENGTH OF FIRST SEGMENT	EAR: DEVELOPMENT OF STERILE SPIKELETS	STERILE SPIKELET - ATTITUDE(MID 1/3 OF EAR)	MEDIAN SPIKELET - LENGTH OF GLUME+AWN cf GRAIN	GRAIN - RACHILLA HAIR TYPE	GRAIN - HUSK	GRAIN - ANTHOCYANIN COLOURATION OF LEMMA NERVES	GRAIN - SPICULATION OF INNER LATERAL NERVES	GRAIN - VENTRAL FURROW - PRESENCE OF HAIRS	GRAIN - DISPOSITION OF LODICULES	KERNEL - COLOUR OF ALEURONE LAYER	SEASONAL TYPE
1	**232**	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
2	**235**	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
3	**278**	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
4	**421**	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
5	**456**	1	1	6	255	5	2	5	3	3	6	1	5	4	5	7	4	5	3	2	2	9	5	1	9	2	1	3
6	**514**	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
7	**578**	8	9	6	1	5	7	5	4	3	3	1	5	5	4	7	5	5	3	2	2	9	4	1	1	2	1	1
8	**674**	2	1	7	1	6	3	5	6	3	5	1	5	5	6	7	3	5	3	2	1	9	4	1	1	2	1	3
9	**688**	4	1	6	5	7	5	3	3	3	5	1	5	5	5	7	3	5	3	2	2	9	4	1	1	2	1	3
10	**750**	3	1	5	1	6	3	4	6	1	4	1	5	5	4	255	6	3	3	1	2	9	4	1	1	2	1	3
11	**806**	5	9	6	1	7	3	6	7	1	5	1	5	5	5	7	5	5	3	2	2	9	6	5	9	2	1	1
12	**816**	7	9	255	1	7	6	255	4	3	4	1	5	5	5	7	5	5	3	2	2	9	1	1	1	2	3	1
13	**820**	5	9	6	1	5	3	5	5	4	5	1	5	5	5	7	5	4	2	2	2	9	3	1	1	2	1	1
14	**884**	7	9	7	1	6	6	7	6	1	5	1	5	6	5	7	4	5	3	2	2	9	5	5	1	2	3	1
15	**899**	7	9	255	1	6	4	255	4	3	5	1	5	4	5	7	3	5	3	2	2	9	1	1	1	2	3	1
16	**904**	5	1	5	3	6	4	4	5	5	4	1	5	4	6	7	3	6	3	2	2	9	5	1	1	2	1	3
17	**947**	4	9	255	3	6	3	255	7	3	5	1	5	5	6	7	5	7	2	3	2	9	1	9	1	255	2	1
18	**951**	7	9	5	1	5	1	6	6	2	5	2	5	2	4	7	3	255	255	255	2	9	8	9	9	2	1	1
19	**954**	7	9	7	1	4	8	5	5	3	5	1	3	6	5	7	5	7	3	2	2	9	5	1	1	2	1	1
20	**994**	7	9	7	1	6	3	5	5	1	4	1	3	5	5	7	6	3	2	2	2	9	7	1	1	2	3	1
21	**1002**	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
22	**1011**	7	1	5	3	5	3	5	5	7	4	1	255	5	7	7	7	3	3	3	2	9	6	1	1	2	1	3
23	**1022**	5	1	6	7	5	5	5	3	5	6	1	3	4	5	7	5	6	3	2	2	9	4	1	1	2	1	3
24	**1029**	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
25	**1031**	4	1	6	3	8	2	3	5	6	6	1	255	3	5	7	5	6	3	3	1	9	3	3	1	2	1	3
26	**1055**	6	9	7	1	7	4	7	6	5	5	1	3	4	5	5	5	6	3	2	2	9	6	1	1	2	1	1
27	**1058**	6	1	9	1	7	4	7	5	4	3	1	3	3	6	3	5	5	3	2	2	9	3	1	1	2	1	1
28	**1071**	7	9	255	1	3	7	255	5	3	5	1	5	7	7	5	5	1	255	2	2	9	1	3	1	2	1	1
29	**1091**	3	1	8	1	5	1	5	5	2	4	1	255	7	5	7	5	7	3	2	2	9	1	1	1	1	1	3
30	**1127**	5	1	7	1	8	2	6	5	6	6	1	3	5	6	5	5	6	3	2	2	9	3	1	1	2	1	255
31	**1133**	5	1	6	3	7	5	4	6	7	3	1	5	4	5	7	4	6	3	2	2	9	3	1	1	2	1	3
32	**1146**	5	1	6	7	6	4	4	5	4	3	1	5	3	5	7	5	6	3	2	2	9	3	5	1	2	1	3
33	**1147**	5	1	6	1	8	5	4	6	5	3	1	5	5	5	7	5	6	3	2	2	9	3	1	1	2	1	3
34	**1160**	7	9	3	1	6	2	3	4	5	5	1	255	6	5	7	5	5	2	2	2	9	2	5	1	2	3	255
35	**1162**	5	9	255	1	7	4	255	5	5	4	1	255	5	5	255	5	5	3	2	2	9	2	5	9	2	1	255
36	**1169**	255	255	255	255	255	255	255	255	255	255	1	255	255	255	255	255	255	255	255	2	255	255	3	1	255	1	1
37	**1170**	4	9	255	1	6	3	3	4	4	5	1	3	5	5	5	4	5	3	2	2	9	255	1	1	2	1	255
38	**1171**	6	9	5	1	6	6	5	6	6	5	1	255	5	5	5	6	6	3	2	2	9	5	3	9	2	1	255
39	**1173**	255	255	255	255	255	255	255	255	255	255	1	255	255	255	255	255	255	255	255	2	255	255	1	1	255	2	1
40	**1183**	6	9	5	5	8	2	6	4	6	6	1	3	5	5	5	5	4	2	2	2	9	3	2	9	2	255	255
41	**1184**	7	9	6	255	8	4	5	5	4	5	1	3	6	5	7	5	4	2	2	1	9	5	3	1	2	3	255
42	**1187**	6	1	6	1	8	4	4	7	2	3	1	5	5	5	7	6	7	3	2	2	9	3	1	1	2	1	3
43	**1188**	5	1	4	3	7	5	3	6	7	5	1	5	5	6	7	5	7	3	2	2	9	3	7	1	2	1	3
44	**1191**	4	1	4	7	8	5	3	7	3	5	1	5	5	5	7	6	7	3	2	2	9	5	5	1	2	1	3
45	**1194**	4	1	5	1	7	5	3	6	5	3	1	5	5	6	7	5	7	3	2	2	9	2	1	1	2	1	3
46	**1196**	7	1	4	255	6	5	4	7	5	4	1	5	5	5	7	5	7	3	2	2	9	1	1	1	2	1	255
47	**1203**	4	1	5	1	8	3	4	6	4	4	1	5	6	5	7	5	7	3	2	2	9	3	1	1	2	1	3
48	**1210**	7	1	8	1	6	4	7	3	6	6	1	5	5	6	5	4	6	2	2	2	9	6	1	1	2	1	255
49	**1211**	7	9	255	1	6	5	255	2	2	5	2	3	5	5	7	4	255	255	2	2	9	255	9	9	2	1	255
50	**1214**	7	9	3	1	8	5	5	3	5	7	2	5	5	6	7	5	255	255	2	2	9	5	9	9	2	1	255
51	**1219**	7	9	5	255	6	3	7	4	6	7	1	3	3	7	3	6	5	2	2	2	9	5	5	1	2	2	255
52	**1221**	7	9	255	1	8	4	255	5	3	5	1	3	7	5	5	4	5	3	2	2	9	1	9	1	2	1	255
53	**1230**	6	9	1	255	6	3	1	1	3	5	2	3	5	5	7	5	255	255	2	2	9	1	9	9	2	1	255
54	**1238**	5	1	6	3	8	4	4	5	4	4	1	5	5	4	7	5	5	3	2	2	9	4	1	1	2	1	3
55	**1242**	5	1	7	1	8	6	5	5	5	4	1	5	6	5	7	6	5	2	2	1	9	4	3	1	2	1	3
56	**1244**	5	1	7	255	7	5	5	5	5	5	1	5	5	5	7	6	6	2	2	2	9	5	1	1	2	255	255
57	**1246**	5	1	7	1	8	5	3	7	4	4	1	255	6	5	7	5	5	3	2	2	9	4	3	1	2	1	3
58	**1256**	6	1	8	255	8	6	4	5	3	3	1	3	7	3	7	5	5	3	2	2	9	1	1	1	2	1	255
59	**1262**	7	9	255	1	7	3	255	3	7	3	1	255	1	5	5	5	5	2	2	2	9	1	1	1	2	1	1
60	**1263**	7	9	6	1	5	4	7	3	6	5	1	3	4	255	5	4	5	3	2	2	9	5	3	1	2	1	1
61	**1264**	7	9	8	1	7	7	5	6	2	3	1	255	5	255	7	5	4	2	2	1	9	8	1	1	2	2	1
62	**1266**	7	9	255	1	8	6	1	3	3	4	1	7	7	255	7	5	7	255	3	1	9	1	7	1	2	255	255
63	**1276**	8	9	5	1	5	6	5	1	2	5	1	255	3	4	5	5	4	1	1	2	9	4	3	9	2	2	1
64	**1281**	7	1	8	1	5	5	6	1	4	5	1	255	5	255	5	5	5	3	2	2	9	8	1	1	2	1	1
65	**1285**	7	9	255	1	7	8	255	4	3	5	1	3	5	5	5	5	5	3	1	2	9	1	1	9	2	1	1
66	**1291**	3	1	5	1	7	2	5	5	4	4	1	255	4	5	7	5	6	2	2	1	9	6	1	1	2	1	3
67	**1293**	3	1	7	1	7	3	6	5	5	6	1	255	4	5	5	6	6	3	2	1	9	6	1	1	2	1	3
68	**1301**	3	1	7	1	7	5	6	5	2	3	1	3	4	4	5	5	6	3	2	2	9	3	1	1	2	1	3
69	**1303**	5	1	6	5	8	6	6	9	3	5	1	3	5	5	7	4	6	2	2	2	9	5	5	1	2	1	3
70	**1311**	3	1	6	1	7	5	5	6	3	2	1	3	4	5	5	5	6	2	2	2	9	6	3	1	2	1	3
71	**1318**	5	9	6	5	8	7	4	7	4	6	1	3	5	6	7	5	5	3	2	2	9	2	5	1	2	3	1
72	**1320**	7	9	2	1	8	5	255	5	6	4	1	5	4	5	7	5	5	1	2	2	9	1	3	1	2	1	1
73	**1326**	7	9	8	1	5	5	6	2	7	5	1	3	3	5	7	7	4	1	2	2	9	6	1	1	2	1	1
74	**1334**	7	9	255	1	8	4	255	5	7	4	1	3	5	4	7	5	6	3	2	1	9	1	9	1	2	1	1
75	**1345**	4	9	8	1	7	7	6	5	5	5	1	3	6	5	5	5	6	2	2	2	9	5	3	9	2	1	1
76	**1350**	4	1	8	3	8	4	6	5	6	7	1	255	3	6	7	5	7	3	2	2	9	5	3	1	2	1	3
77	**1392**	5	9	8	5	6	3	5	5	5	6	1	3	4	5	7	6	5	2	2	2	9	3	3	1	2	3	1
78	**1397**	7	9	1	4	7	3	255	6	3	5	2	5	4	6	6	4	255	255	3	2	9	1	8	9	2	1	2
79	**1402**	5	1	255	3	8	3	255	3	6	7	1	3	4	6	5	6	2	1	2	2	9	1	3	1	2	1	1
80	**1404**	5	9	255	5	8	2	255	4	6	6	1	3	3	6	5	5	1	1	2	2	9	1	3	1	2	3	1
81	**1409**	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255	255
82	**1410**	6	1	6	5	7	6	4	6	4	5	1	3	3	5	7	5	6	3	2	1	9	4	7	1	2	1	3
83	**1411**	5	1	7	6	7	5	6	6	4	4	1	3	4	5	7	5	6	3	2	1	9	6	1	1	2	2	3
84	**1415**	5	1	6	3	8	6	4	6	5	6	1	3	3	6	7	5	6	3	2	2	9	5	1	1	2	1	3
85	**1419**	3	1	6	1	9	2	8	4	3	3	1	3	4	4	7	5	7	3	2	2	9	1	1	1	2	255	3
86	**1423**	4	1	6	3	6	5	5	6	4	6	1	3	3	5	5	5	6	3	2	1	9	5	1	1	2	1	3
87	**1427**	4	1	5	3	7	6	5	6	4	7	1	3	3	7	5	5	6	3	2	1	9	4	1	1	2	1	3
88	**1431**	5	1	7	5	7	7	5	6	5	7	1	3	4	5	7	3	6	3	2	2	9	4	5	1	2	1	3
89	**1435**	4	9	5	6	7	5	3	8	4	5	1	5	6	7	3	5	4	2	2	2	9	2	2	1	2	3	1
90	**1442**	7	9	255	7	8	7	255	5	4	5	1	3	5	5	6	5	2	1	2	2	9	1	3	1	2	1	1
91	**1443**	6	9	9	2	8	4	5	5	6	6	1	4	4	5	7	5	5	3	2	2	9	5	4	1	2	1	1
92	**1445**	7	9	255	3	7	3	255	6	5	5	1	3	3	6	6	7	5	2	2	2	9	1	9	1	2	1	1
93	**1457**	7	9	4	1	8	5	2	5	4	4	2	5	4	4	5	5	255	255	2	2	9	3	7	9	2	3	1
94	**1463**	7	9	2	1	7	5	2	5	3	3	2	3	4	5	7	3	255	255	3	2	9	3	8	9	2	1	1
95	**1466**	8	9	255	5	7	6	255	3	6	6	1	3	4	6	5	5	2	1	2	2	9	1	1	1	2	1	1
96	**1468**	9	9	8	4	7	7	5	2	6	5	1	3	5	5	7	5	1	255	2	2	9	7	1	9	2	1	1
97	**1471**	7	9	3	5	7	4	255	5	7	4	1	5	5	5	5	4	1	255	2	1	9	5	1	1	2	255	1
98	**1474**	3	1	5	3	5	5	5	4	3	4	1	3	4	5	7	5	5	3	2	2	9	4	7	1	2	2	3
99	**1485**	5	1	7	4	6	5	5	6	4	4	1	3	4	5	7	5	2	1	2	2	9	5	1	1	2	3	3
100	**1492**	5	1	7	1	8	8	4	5	4	3	1	3	5	5	7	4	5	3	2	2	9	4	1	1	2	1	3
101	**1501**	5	1	5	3	6	6	4	5	4	5	1	5	4	6	7	5	6	3	2	2	9	5	1	1	2	1	3
102	**1511**	7	9	7	5	7	7	6	6	6	4	1	3	4	5	7	5	5	3	1	2	9	7	4	1	2	1	1
103	**1528**	7	9	6	5	8	7	4	7	5	4	1	3	3	5	7	5	1	255	1	2	9	8	3	9	2	1	1
104	**1534**	4	9	255	5	6	5	255	6	6	6	2	5	3	5	4	3	255	255	2	2	9	255	8	9	2	1	1
105	**1535**	5	9	255	4	8	5	255	7	5	6	2	4	3	5	5	3	255	255	3	2	1	255	7	1	2	1	1
106	**1540**	8	9	255	7	8	6	255	7	6	4	1	3	4	5	5	5	5	2	2	2	9	1	3	1	2	1	1
107	**1555**	4	1	5	4	7	5	5	5	6	5	1	3	2	6	6	4	6	3	2	2	9	5	1	1	2	1	3
108	**1566**	3	1	7	2	7	3	5	6	5	4	1	3	1	7	4	5	7	3	2	2	9	5	3	1	2	1	3
109	**1567**	3	1	8	3	7	3	5	6	4	4	1	3	5	5	7	5	7	3	2	2	9	4	7	1	2	1	3
110	**1572**	3	1	6	1	7	7	5	5	2	3	1	3	4	5	7	5	6	3	2	2	9	5	1	1	2	1	3
111	**1575**	4	1	4	2	7	5	5	5	5	3	1	3	5	5	7	5	7	3	2	2	9	5	1	1	2	1	3
112	**1576**	5	1	6	2	7	5	5	5	5	5	1	3	3	6	5	5	6	3	2	2	9	5	3	1	2	1	3
113	**1579**	255	1	255	255	255	255	255	255	255	255	1	255	255	255	255	255	255	255	255	2	255	255	255	1	255	255	3
114	**1580**	5	1	8	3	7	5	5	5	5	4	1	3	5	5	7	5	7	3	2	2	9	6	5	1	2	1	3
115	**1589**	5	1	6	1	7	6	5	5	4	4	1	3	3	6	6	5	6	3	2	2	9	5	1	1	2	1	3
116	**1591**	3	1	7	4	7	5	5	5	5	5	1	3	3	6	7	5	6	3	2	2	9	5	9	1	2	1	3
117	**1597**	7	1	6	1	7	5	6	1	4	4	1	3	3	5	7	5	7	3	2	2	9	5	3	1	2	1	3
118	**1599**	7	9	8	3	8	7	6	7	5	3	1	3	4	5	7	4	6	3	2	2	9	6	5	1	2	1	1
119	**1604**	6	9	255	2	8	7	255	5	6	3	1	3	3	6	7	5	5	3	2	2	9	1	5	1	2	1	1
120	**1606**	6	9	255	1	8	5	255	4	6	4	1	3	4	5	7	4	6	3	2	2	9	1	1	1	2	1	1
121	**1608**	7	9	255	2	7	4	255	1	6	3	1	3	3	5	7	5	5	1	2	2	9	1	5	1	2	3	1
122	**1626**	7	9	3	1	9	7	6	7	5	5	2	3	5	4	7	5	255	255	2	2	9	4	9	9	2	1	1
123	**1628**	7	9	4	5	5	5	5	3	5	2	1	3	6	4	7	5	5	2	2	2	9	1	2	1	2	2	1
124	**1668**	5	1	6	1	7	6	6	5	5	4	1	3	5	5	7	3	1	255	1	2	9	3	1	1	2	1	3
125	**1669**	4	1	6	1	7	6	5	7	5	4	1	3	3	6	7	3	1	255	2	2	9	2	1	1	2	1	3
126	**1697**	7	9	255	1	8	3	255	7	3	4	1	3	4	6	7	7	6	3	2	2	9	1	3	1	2	2	1
127	**1700**	7	9	7	6	4	4	7	3	5	7	1	4	5	7	7	3	7	3	2	2	9	4	3	1	2	1	1
128	**1705**	7	9	255	5	7	5	255	3	4	4	1	3	3	6	6	3	2	255	2	2	9	1	5	9	2	1	1
129	**1717**	6	9	4	1	6	5	4	6	4	5	2	4	5	4	7	3	255	255	2	2	9	3	7	9	2	1	1
130	**1726**	7	9	255	7	7	5	255	2	4	5	1	3	6	6	6	4	2	255	2	2	9	1	6	1	2	1	1
131	**1728**	6	1	8	4	8	5	6	4	5	4	1	3	3	7	4	3	5	3	2	2	9	5	4	9	2	1	1
132	**1741**	6	1	8	1	7	6	5	6	5	4	1	3	5	5	7	5	5	3	2	2	9	5	1	1	2	1	3
133	**1745**	5	1	9	5	8	6	3	5	8	6	1	3	5	6	7	5	7	3	2	2	9	5	1	1	2	1	3
134	**1746**	5	1	9	4	7	5	5	6	7	5	1	3	4	5	7	5	7	3	2	1	9	5	1	1	2	1	3
135	**1769**	4	1	8	2	7	5	5	6	4	4	1	3	3	5	7	5	6	3	2	2	9	5	1	1	2	1	3
136	**1780**	255	255	255	255	255	255	255	255	255	255	1	255	255	255	255	255	255	255	255	2	255	255	255	1	255	255	3
137	**1825**	6	9	3	3	6	2	3	3	4	5	1	3	4	7	6	5	5	3	2	2	9	3	4	9	2	1	1
138	**1841**	3	1	7	5	7	6	6	4	5	4	1	4	5	6	6	5	7	3	2	2	9	5	1	1	2	1	3
139	**1862**	3	1	7	3	7	6	5	7	5	4	1	4	4	6	7	4	6	3	2	1	9	5	1	1	2	1	3
140	**1864**	2	1	8	5	6	6	6	6	6	5	1	4	5	6	7	5	7	3	2	1	9	5	1	1	2	1	3
141	**1865**	3	1	7	3	8	6	6	5	4	4	1	4	4	6	6	5	7	3	2	2	9	6	1	1	2	1	3
142	**1867**	7	1	7	3	7	7	4	4	4	5	1	4	5	6	7	5	7	3	2	1	9	5	1	1	2	1	3
143	**1871**	6	1	9	3	7	9	6	7	6	5	1	5	2	6	6	6	1	255	3	2	9	6	1	9	2	1	3
144	**1880**	5	9	255	1	6	5	255	1	4	4	1	5	5	5	5	4	1	255	2	2	9	1	4	9	2	3	1
145	**1886**	6	9	255	1	6	6	255	5	4	7	2	5	6	6	6	3	3	255	3	2	9	1	7	9	2	1	1
146	**1892**	6	9	255	1	5	1	255	4	4	5	1	5	4	5	5	4	3	1	2	2	9	1	2	1	2	1	1
147	**1902**	6	9	255	1	7	4	255	4	6	5	1	5	5	5	5	6	5	2	2	2	9	1	3	1	2	1	1
148	**1919**	5	9	255	2	6	4	255	5	6	5	1	5	4	6	5	5	5	3	3	1	9	1	5	1	2	1	1
149	**1923**	7	9	6	1	5	7	6	6	4	6	1	5	5	5	6	5	5	255	2	2	9	5	5	1	2	1	1
150	**1939**	6	1	7	1	7	4	5	4	6	6	1	5	5	5	7	5	5	3	2	1	9	3	5	1	2	1	3
151	**1940**	5	1	7	1	5	5	6	4	4	5	1	5	5	5	6	5	6	3	2	2	9	4	3	1	2	1	3
152	**1951**	4	1	7	1	8	4	5	4	5	5	1	5	4	5	7	5	5	3	2	2	9	4	3	1	2	1	3
153	**1963**	4	1	7	3	5	5	5	4	5	5	1	5	6	6	7	5	5	3	2	2	9	5	3	1	2	1	3
154	**1966**	3	1	6	2	6	5	6	5	3	4	1	5	4	5	7	5	6	3	2	1	9	4	1	1	2	1	3
155	**1974**	3	1	7	4	7	5	6	4	5	6	1	5	5	6	6	4	6	3	2	2	9	4	1	1	2	1	3
156	**2003**	7	9	5	4	6	4	6	5	4	5	2	5	4	5	6	4	255	255	255	2	9	1	7	9	2	3	1
157	**2025**	4	1	7	1	6	6	4	6	5	6	1	5	5	3	7	3	255	3	2	2	9	5	1	1	2	2	3

Table 4. DUS phenotypic database for the 28 traits currently scored under DUS assessment of barley, across the 169 varieties included in the study. Missing data = 255. The number of missing entries and percentage fill for each trait is indicated.

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**

Table 5. Predictive value of a subset of the genetic markers relevant to DUS traits. Good phenotypic predictions (> 92 %) are obtained by molecular markers for eight DUS traits.

[1] SNP C associated with scores 1-4 (erect – semierrect/intermediate), Del associated with scores 6-9 (intermediate/semiprostrate – prostrate. Varieties with score 5 (intermediate) were removed from the analysis.

[2] In LD with causative InDel. SNP T predictive of score 1 (absence of anthocyanin), SNP C predictive of scores 2-9 (increasing presence)

[3] causative SNP (1 of 3)

[4] Haplotype

[5] not causative, different locus: row number ideotype.

[6] 6-row varieties excluded from analysis. SNP G predictive of scores 1 (parallel) and 2 (parallel-divergent), SNP C predictive of score 3 (divergent)

[7] SNP A predictive of score 1 (absent/v weak), SNP C predictive of score 2-9 (v weak – v strong)

[8] SNP G predictive of score 1 (absent/v weak), SNP A predictive of score 2-9 (v weak – v strong)

[9] Causative locus. Only 1 example of frontal 'bib' type lodicule disposition (score 1)

[10] Causative locus. Only 1 example of frontal 'bib' type lodicule disposition (score 1). No polymorphism in UK lines assayed
[11] SNP C predictive of score 1 (white), SNP T predictive of scores 2-3 (weakly - strongly coloured)
[12] SNP G predictive of score 1 (white), SNP A predictive of scores 2-3 (weakly - strongly coloured)
[13] SNP C predictive of score 1 (white), SNP A predictive of scores 2-3 (weakly - strongly coloured)
[14] VRN-H1 multiplex PCR assay
[15] Note: heterozygotes ignored. Not diagnostic, as SNP A can confuse winter haplotype 1A with spring haplotype 1B, and wrongly predicts winter haplotype 5C
[16] Haplotype. As for HvVRNH1_SNP2, but with 'Del' diagnostic for winter haplotype 5C. Will not discriminate between winter haplotype 1A and spring haplotype 1B

James Cockram, Carol Norris, Donal O'Sullivan, NIAB, UK

[Annex follows]

ANNEX

Annex

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

50	Labea	Winter	2
51	Lina	Spring	2
52	Logan	Spring	2
53	Malta	Winter	2
54	Marinka	Winter	2
55	Maris Otter	Winter	2
56	Mehola	Winter	2
57	Meltan	Spring	2
58	Nudinka	Spring	2
59	Nure	Winter	2
60	Optic	Spring	2
61	Panda	Winter	2
62	Pastoral	Winter	2
63	Pearl	Winter	2
64	Pioneer	Winter	2
65	Pipkin	Winter	2
66	Plaisant	Winter	6
67	Prisma	Spring	2
68	Proctor	Spring	2
69	Puffin	Winter	2
70	Ragusa	Winter	6
71	Regina	Spring	2
72	Scarlett	Spring	2
73	Sergeant	Spring	2
74	Sonja	Winter	2
75	Spratt Archer	Spring	2
76	Tipple	Spring	2
77	Tocada	Spring	2
78	Torrent	Winter	2
79	Tremois	Spring	2
80	Tria	Winter	2
81	Triumph	Spring	2
82	Vada	Spring	2
83	Vanessa	Winter	2
84	Villa	Spring	2
85	Vogels Gold	Winter	6
86	Volla	Spring	2
87	Waggon	Spring	2
88	Warboys	Winter	2
89	Etu	Spring	6
90	Morex	Spring	2

Annex 1. Barley varieties selected for the validation panel

[End of Annex and of document]