E

BMT/9/9
ORIGINAL: English
DATE: June 1, 2005

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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

Ninth Session
Washington, D.C., June 21 to 23, 2005

SNPS IN BARLEY: A POTENTIAL "OPTION 1" APPROACH

Document prepared by experts from the United Kingdom

SNPS IN BARLEY: A POTENTIAL 'OPTION 1' APPROACH

Donal O'Sullivan, Elena Chiapparino, Konstantina Stamati, Manesh Chatterjee, Paolo Donini, David Lee, James Cockram, Paolo Donini, David Laurie* and Robert J Cooke
NIAB Cambridge UK and * John Innes Centre Norwich UK

Introduction:

1. At the eighth session of the BMT meeting, held in Tsukuba, Japan, from September 3 to 5, 2003, NIAB presented data on the analysis of 132 barley varieties using five single nucleotide polymorphism (SNP) markers:

Locus	**Chromosome**	**Polymorphism[a]**	**Ratio of allelic compositions[b]**
MWG2062	7H	R	80 G : 50 A : 2 H
ABC465	7H	Y	123 T : 9 C
MWG2218	6H	S	82 C: 50 G
MWG502	5H	R	78 A :54 G
ABG601	4H	Y	89 C : 40 T : 3 H

[a] R =A,G; Y =C,T; S =G,C
[b] H = heterozygote

2. In summary:

- the five loci analysed had individual separation coefficients of between 0.12 - 0.49;
- the 132 varieties could be separated into 20 groups (with 1 - 16 varieties);
- the overall separation rate was 98% (criterion = one locus difference);
- ABC465 showed strong bias towards the T allelic form – possibly related to selection for the sucrose synthase gene
- the allelic status of ABG601 correlated to winter/spring character – possibly due to linkage with the *Sgh1*(=*VRN-H2*) locus (and therefore vernalisation).

3. In further work and as part of a continuation of the EU-funded Gediflux project, we have been investigating the possibility of developing a set of SNPs that could be used to assess the vernalisation characteristic in barley.

Molecular Basis of Vernalisation:

4. Genetic studies have shown that variation at two major gene loci in the cereal vernalisation pathway underlies the differing vernalisation requirement of spring versus winter cereals. The loci, designated *VRN1* and *VRN2*, have been comparatively mapped and orthology between *VRN-A^{m}1* from einkorn wheat (*T. monococcum*), *VRN-A1*, *VRN-B1*, *VRN-D1* from the homeologous genomes of hexaploid wheat (*T. aestivum*), *VRN-R1* from rye (*S. secale*) and *VRN-H1* from barley (*H. vulgare*) from group 5 chromosomes was demonstrated. Similarly, *VRN-A^{m}2*, *VRN-A2*, *VRN-B2*, *VRN-D2* and *VRN-H2* from group 4 chromosomes are orthologous. Both *VRN-A^{m}1* and *VRN-A^{m}2* have been positionally cloned from *T. monococcum*, opening the way to a detailed molecular understanding of the vernalisation requirement in cereal crops. The proposed model for the action of these genes is that *VRN-A^{m}2* is a repressor of *VRN-A^{m}1*. Spring forms can originate either by mutation of

VRN-A^{m}2 (removing the repressor) or by mutation of *VRN-A^{m}1* (removing its ability to recognise the repressor).

Background Results from Gediflux:

5. Part of the research within Gediflux was concerned with analysis of the *Bmy1* locus (ß-amylase), which is linked to *VRN-H2* on chromosome 4H. For *Bmy1*, a triplex SNP assay was developed, and 467 European varieties were analysed. This resulted in four haplotypes (known as Sd2L, Sd1, CTC, and Sd2H), one of which was found predominantly in winter types. Portions of *VRN-H1* were sequenced from a panel of 21 varieties, leading to the identification of 5 SNPs, which divided the varieties into four haplotypes, one of which contained only winter varieties.

Proposed Research:

6. The hypothesis to be tested was thus that specific haplotype combinations at the *VRN-H1* and *VRN-H2* loci, which can be rapidly identified via SNP analysis, will be diagnostic for seasonal growth habit.

7. The significance of this would be the opportunity to develop an Option 1- type approach to the use of molecular markers in DUS testing. One of the characteristics used in DUS testing of cereals is the winter/spring growth habit and vernalisation requirement. In barley, the current UK protocol for evaluating seasonal growth habit stipulates that –

> *"In the case of winter varieties an additional 50 ear-rows are sown in late April during the first year of tests to examine the uniformity of the vernalisation response of the variety under test."*

8. The scoring of this characteristic therefore entails a dedicated planting of 50 ear rows per candidate variety, as well as the inclusion of example varieties; these plots (50 ear rows per winter variety) require normal husbandry over the growing season and observation and scoring are completed only when the latest flowering spring variety is fully mature (GS91/92). A molecular test for vernalisation, which can be accomplished quickly and cost-effectively, thus has attractions as a direct replacement for an existing field-based characteristic and in addition might serve as a model for the use of molecular markers in other crops.

9. The presentation will highlight some of the very recent results from the project.

[End of document]