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

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ADDENDUM
FUNCTIONAL SNP MARKERS FOR THE VERNALIZATION REQUIREMENTS IN BARLEY: AN OPTION 1 APPROACH

Document prepared by experts from the United Kingdom
Functional SNP Markers for the Vernalization Requirement in Barley

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Talk Outline:

- Project background
- Results so far
- Questions

Seasonal Growth Habit (SGH) – major phenotypic division in barley

Spring barley

Winter barley
SGH – UPOV TG/19/10 characteristic 29

SGH states:
1 = spring
2 = alternative
3 = winter

Genetic control of SGH

- Two major loci in European barley (VRN-H1 and VRN-H2)
- VRN-H1 responsible for flowering mechanism
- VRN-H2 represses VRN-H1
- During cold treatment repression is removed
- Mutation in either gene = spring type
VRN-H2 represses VRN-H1

VRN-H1 not expressed, preventing floral transition

Flowering

Winter variety before cold treatment

VRN-H2 represses VRN-H1

Removal of repression allows VRN-H1 expression

Flowering

Winter variety after cold treatment
**VRN-H1**: 6 spring, 3 winter alleles

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**Aims**

- UPOV option 1 approach – “Molecular characteristics as a predictor of traditional characters
- Investigate ‘Alternative’ lines
- Detect SGH off types
New *VRN-H1* assay + *VRN-H2* assay

100 UK varieties recorded on the UK NL/RL 1991-2007 (50W, 50S)

Combined haplotypes predict SGH in all 100 varieties

\[ \text{VRN-H1 assay} + \text{VRN-H2 assay} = \text{predicted SGH} \]

Generated a reference database of *VRN-H1* and *VRN-H2* alleles

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**Additional work...**

- Now single well markers for *VRN-H1* and *VRN-H2* have been designed and validated:
- Use vernalization field data from 2 years
- Test and sequence ‘Alternative’ lines
- Develop PCR marker to detect and quantify off-types (uniformity)
Genotyping ‘alternative’ varieties

Apart from ‘Damas’, all alternative lines looked at have unique haplotypes. This suggests:

1. VRN-H1 is likely to control the ‘alternative’ SGH’ phenotype
2. Their complete gene sequences will contain polymorphisms that will easily allow them to be uniquely identified
Detection of off-types

(1) Optimisation of primers
(2) Determine primer sensitivity

Minimum sensitivity equates to 1 off-type in 400

Conclusions to date

- Progress towards explaining the ‘alternative’ growth habit has been promising, identifying 7 novel VRN-H1 haplotypes
- The positions of major intron I deletions have been identified by PCR analysis
- Full length sequencing of VRN-H1 in ‘alternative’ types is ~70% complete. Once finalised, this will allow deployment of appropriate diagnostic assays in the detection of off-types.
- Primers for detection of off-types have been optimised and their sensitivity determined using serial DNA dilutions.
- The reference database of VRN-H1 and VRN-H2 alleles has been updated with the results of the single-well multiplex VRN-H1 assay, as well as the inclusion of genotypes for an additional sixteen varieties.
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