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FUNCTIONAL SNP MARKERS FOR THE VERNALIZATION REQUIREMENT IN BARLEY: A POTENTIAL 'OPTION 1' APPROACH

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Functional SNP Markers for the Vernalization Requirement in Barley: A Potential 'Option 1' Approach

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1. Introduction

In this work we investigated the possibility of developing a gene-based molecular marker assay that could be used to assess the vernalization characteristic in barley. For DUS purposes, the scoring of this characteristic currently involves planting out significant areas of submitted winter varieties in springtime in order to confirm their seasonal growth type. Winter varieties do not flower at all in these circumstances and since the plants do not produce heads or grains, the trial is not used to score any other characteristics. A molecular test for vernalization, which can be accomplished quickly and cost-effectively, has attractions as a direct replacement for the existing field-based characteristic and in addition might serve as an "Option 1"* model for the use of molecular markers in other crops and for further work on barley.

2. Molecular basis of vernalization in cereals

Genetic studies have shown that variation at two major gene loci in the cereal vernalization pathway underlies the differing vernalization requirement of spring versus winter cereals. The loci, designated *Vrn1* and *Vrn2*, have been comparatively mapped to the group 5 chromosomes and orthology between *Vrn-A^m1* from einkorn wheat (*T. monococcum*), *Vrn-A1, Vrn-B1, Vrn-D1* from the homoeologous genomes of hexaploid wheat (*T .aestivum*), *Vrn-R1* from rye (*S. cereale*) and *Vrn-H1* from barley (*H. vulgare*) has been demonstrated. Similarly, *Vrn-H2* and *Vrn-A^m2* are orthologous, although an A genome-specific translocation means that they are found on chromosomes *4HL* and *5A^m*L respectively. Both *Vrn-A^m1* and *Vrn-A^m2* have been positionally cloned from *T. monococcum*, opening the way to a detailed molecular understanding of the vernalization requirement in cereal crops. The proposed model for the action of these genes is that *Vrn- A^m2* is a repressor of *Vrn- A^m1*. Spring forms can originate either by mutation of *Vrn-A^m2* (removing the repressor) or by mutation of *Vrn-A^m1* (removing its ability to recognize the repressor).

3. Objectives addressed

1. To characterize sequence diversity in the *Vrn-H1* gene in European barley varieties and to assess the association between polymorphism's at *Vrn-H1* and *Vrn-H2* loci and seasonal growth habit
2. To develop an Option 1-type approach, by testing whether specific haplotype combinations at the *Vrn-H1* and *Vrn-H2* loci can be rapidly and accurately identified using low-cost PCR assays. The ultimate aim is to develop a single reaction PCR assay which is predictive for seasonal growth habit.
3. To make recommendations for implementation of vernalization markers in future DUS testing.

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* Option 1(a): Use of molecular characteristics which are directly linked to traditional characteristics (gene specific markers) – see documents TC/38/14-CAJ/45/4 and TC/38/14 Add.-CAJ/45/5 Add.)

4. Summary of Results

- Firstly, the full sequence of *Vrn-H1* was obtained from a selection of barley varieties thought to contain different alleles or forms of the gene, so that the precise sequence differences between one form and another could be revealed. We observed a single invariant allele or haplotype of the *Vrn-H1* locus in all true vernalization-requiring winter varieties studied.
- Furthermore, a number of spring alleles of the *Vrn-H1* locus were characterized in detail and all could be identified rapidly and distinguished from the canonical winter allele in a single reaction duplex PCR assay based on characteristic insertion/deletion polymorphisms.
- We validated the test by checking the predicted growth type status of 48 winter and 48 spring barley varieties whose growth type had been formally evaluated as part of DUS testing carried out at NIAB. All 96 varieties were correctly classified by the test.
- We also developed a test that revealed spring forms of the *Vrn-H2* gene because, in theory, the spring form of *Vrn-H2* could independently cause a variety to assume the spring growth type. However, in practice, spring forms of *Vrn-H2* only occurred in varieties already identified as possessing spring forms of the *Vrn-H1* gene, and never alone as the sole cause of loss of vernalization requirement.

5. Conclusions

The PCR test developed here, that will identify all known spring alleles at the *Vrn-H1* locus and major deletion polymorphism at the *Vrn-H2* locus, can be performed within days of receipt of a candidate variety, thus saving time and allowing any problems to be verified later by sowing if necessary. This is the first example of which we are aware where a DNA test based on a specific gene difference underlying alternative states of a DUS characteristic has been successfully developed. This is highly significant because previous research has generally emphasized the development of approaches employing 'anonymous' DNA markers to reflect general levels of relatedness/distinctness between varieties and uniformity and stability within a variety (Option 2 and/or 3 approaches). It is probable that in the long term, Option 1 type approaches to the use of molecular markers in DUS testing will prove to be more profitable.

6. Further work

Several new perspectives flow from this work. Now that a qualitative test that predicts the seasonal growth type is available, some additional work is needed to establish a procedure to detect and quantify the percentage of off-types in an unknown sample (i.e. uniformity). The application of the molecular test in assessment of uniformity would require modification of the assay to a co-dominant form and validation of the sensitivity and linearity of the response of the modified assay. Also, a reference database of *Vrn-H1* and *Vrn-H2* alleles found in varieties of 'common knowledge' would be needed. Both these aspects could be advanced by introducing the use of the seasonal growth type diagnostic test to run in parallel with the traditional vernalization trial for a number of seasons, so that the size of the reference database could be incrementally increased and that uniformity levels expressed in the field could be compared with the level of off-types observed using the molecular test.

In the longer term, we anticipate that in the coming years a number of other morphological traits will be explained in terms of precise differences in DNA sequence between varieties (for instance, the row number characteristic in barley) and that when molecular description of a number of characteristics together becomes possible, the efficiency of DNA profiling as a major component of DUS assessments will become ever more apparent.

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