Technical Working Party on Testing Methods and Techniques   TWM/2/5

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UNIFORMITY ASSESSMENT USING MOLECULAR MARKERS

Document prepared by experts from the United Kingdom

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INTRODUCTION

1.   Uniformity is assessed on the basis of phenotypic characteristics (document TG/1 "General Introduction to the Examination of Distinctness, Uniformity and Stability").

2.   We propose a way to assess genetic variability in plant varieties. Whilst this cannot be used to assess uniformity directly, it could be a useful supplementary method. The approach is particularly designed for cross-pollinated crops where plants from a variety are not expected to be identical.

3.   This development arises from INVITE, a collaborative project between European Examination Offices and researchers. This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 817970.

The approach

4.   Firstly, we define a measure of genetic variability. For SNP-like markers, AA corresponds to 0, AB to 0.5, and BB to 1, where the numbers 0, 0.5 and 1 represent the relative frequency of the B allele at a SNP. In a sample of plants from a diploid variety, we may have a mixture of AA, AB and BBs at the SNP, corresponding to a mixture of 0, 0.5 and 1. So we calculate the variance of these numbers for the SNP, then average these variances over all the SNPs. This gives a measure of genetic variability.

5.   Genotyping individual plants is more expensive than genotyping a pooled sample from the variety. With methods such as Genotyping-by-Sequencing (GBS), or Sequence Capture, it is possible to estimate the allelic frequency in the pool. This will produce a number between 0 and 1 in a diploid, representing the average frequency of the B allele in the pool. This is the method developed in INVITE for genotyping perennial ryegrass varieties.

6.   An approximation has been derived for calculating the genetic variability based on a single pooled sample. It is not possible to produce an exact estimate of the genetic variability due to loss of information from the pooling, but we see in practice that the approximation works surprisingly well. The approximation will be explained further in the presentation.

In practice

13.   We have evaluated the method using two perennial ryegrass data sets. Results will be demonstrated in the TWM session.

14.   We found that the approximation method has potential to be useful in practice, though further work (outside of INVITE) is needed. The approximation largely preserves the ordering of varieties in terms of their variability. We expect it to work better in diploids than crops with higher ploidies.

15.   Although the method has been developed for cross-pollinated crops, it may also have potential for other crops, as long as the marker method estimates allelic frequencies in pools.

Potential uses of the method

6.   In the DUS context, the method could be considered as a supplementary indicator for uniformity. In the main, this will be "free" extra information if the variety is genotyped for reference collection management. In particular, we suggest the following cases:

   a) As an early indicator of potential problems, both for candidates and when (re)stocking reference collections.
   b) As supplementary information in uniformity assessment, e.g., to confirm uniformity problems or when there are few comparable reference varieties. Further consideration could be given as to how the method could be adapted for different methods of uniformity assessment.

7.   In crop and seed quality assessment post-registration (ISTA, OECD) for assessing varietal purity. The tool may be used to reduce the laboratory or field inspection work required, through a screening process.

8.   By plant breeders and seed producers for quality control.

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