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BMT-TWA/Wheat/2/2
ORIGINAL: English
DATE: June 16, 2004

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR WHEAT

Second Session
Poznań, Poland, June 28, 2004

A REVIEW OF UK RESEARCH ON THE USE OF MOLECULAR MARKERS FOR WHEAT DUS TESTING

Document prepared by experts from the United Kingdom

1. The results of a research programme on the possible uses of molecular markers in wheat DUS testing have been reported previously (e.g. eighth session of the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT), held in Tsukuba, Japan, from September 3 to 5, 2003). This paper summarises the main conclusions from that work and outlines future plans.

2. The UK research programme was centred on the development of a "test set" of DNA microsatellite (simple sequence repeat, SSR) primer pairs in wheat. A preliminary screen of 55 markers (mostly from the IPK programme) produced an initial set of 23 SSR primer pairs, which were used to analyse 20 individuals from each of 10 varieties. A sub-set of 8 markers, chosen for their ease and reliability of scoring as well as their PIC values and levels of "uniformity" within varieties, was then used to analyse 48 individuals from a further 30 varieties. We reported in 2003 (BMT, Japan):

(i) the outcome of this uniformity assessment: although varieties varied in their levels of uniformity at the SSR loci, the overall mean level of uniformity was 93%.

(ii) a comparison with storage proteins and phenotypic data in terms of the separation coefficients: the 8 SSRs gave 99.7% separation, compared to 92.1% (3 HMW glutenin loci), 95.7% (5 gliadin 'loci') and 100% (26 UPOV characteristics). Remember that this needs to be set against the time taken – two whole seasons for the phenotypic approach from start to finish, compared to approximately 1 week (SSRs) or 2-3 days (proteins).

(iii) an analysis of successive generations of varieties (i.e. an examination of aspects of stability): there were effectively no major issues with the stability of varieties at the SSR loci.

(iv) a comparison with some field-based observations, which showed that in some cases it was possible to confirm 'off-types' identified in the field using SSRs.

3. One conclusion from this was that there would seem to be no reason why such well characterised and tested SSRs could not be used for DUS testing. We have already suggested a revised approach to DUS testing in wheat using SSRs (an Option 3 approach – see document TC/38/14-CAJ/45/5), which could offer reductions in time and costs and/or form the basis of a means of managing large reference collections more effectively, without undermining current levels of protection. The results outlined above specifically addressed the concerns raised by some members of the Review Group that Uniformity (U) and Stability (S) aspects were not being examined.

4. However, given the current view within UPOV that there are fewer difficulties with Option 2 approaches, we also extended the number and range of SSRs used to analyse this set of varieties, and investigated the correlation between various measures of 'genetic' and 'phenotypic' distance. First results from this exercise were also reported in Japan, and generally showed only modest correlations between the distance estimates. One of the more promising approaches was the use of the software PREDIP, carried out in collaboration with our colleagues at GEVES. This is a statistical predictive method, which needs a 'learning' set of varieties to take into account phenotypic and molecular variability and to design the links between the data types. In the practical DUS testing case, this learning set would be the reference collection. With the UK wheat data, the sample size (40 varieties) was too small to work with effectively using qualitative data and thus the data were considered as if they were quantitative. Also, seven characteristics that did not exhibit sufficient variability had to be omitted. Dendrograms were constructed from the SSR data (using Nei and Li distance), from the phenotypic data (Euclidean) and from the predicted phenotypic distance, and the resultant groupings compared. Whilst the correlation between Nei and Li distance and phenotypic distance was low (0.36), that between the predicted and observed phenotypic distances was much better (0.89). It has to be remembered, however, that this is not a validation test of the method, as the same data are being used for the learning set and for the test set. Nonetheless, these initial results were encouraging.

5. We now have access to a much enlarged set of data on wheat varieties, comprising 856 varieties (spring and winter) analysed at 42 SSR loci. Although it will not be possible to collate all of the necessary morphological data for all of these varieties, we will be able to select a sufficient number (in excess of 200) in order to undertake a more thorough analysis of PREDIP. We thus hope that in further collaboration with GEVES, we will be able to make progress with investigating an Option 2 approach for wheat DUS testing. This will then in turn enable us to compare the relative merits of Option 2 and Option 3 approaches.

6. Finally, NIAB is currently undertaking a Defra-funded desk study on molecular markers for ‘functional ‘ (e.g. VCU-related) characteristics across a range of species, including wheat. The outputs of this will be: (i) a survey of existing markers for traits; and (ii) a list of those traits where there is a perceived need to develop markers. Such markers will be important in a range of ways, such as the more targeted use of marker-assisted breeding approaches and the measurement of functional genetic diversity. However, they could also form the basis for Option 1-type approaches, if validated in the DUS context. In the longer term, this may represent the best route for the application of molecular markers in DUS testing – the use of specific markers linked to (or part of the genes coding for) characteristics which enable those characteristics to be assessed more cost-effectively.

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