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BMT/10/11
ORIGINAL: English
DATE: October 27, 2006

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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

Tenth Session
Seoul, November 21 to 23, 2006

A RESEARCH PROJECT CO-FINANCED BY CPVO:
"MANAGEMENT OF WINTER OILSEED RAPE REFERENCE COLLECTIONS"

Document prepared by experts from the United Kingdom

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1. Introduction

Although varying approaches to the DUS testing of winter oilseed rape (WOSR) are taken within Europe, there are a number of problems common to all of the countries where WOSR is a major crop:

- *The size of the field trials.* The number and type of candidate varieties entered for DUS testing in EU member states (MS) is increasing annually, which causes an increase in both the size and the complexity of the DUS field trials (e.g. because of the need to grow both parental and maintainer lines of hybrids in trials);.
- *The size of the reference collection.* The increasing number of WOSR varieties (including hybrids of various types), the enlargement of the European Union and the requirement to compare new varieties with those whose existence is a matter of "common knowledge" at the time of application have all contributed to a substantial increase in the size of the variety reference collection which should be included in DUS trials.

In principle, every year newly listed varieties from all member States (MS) should be added to each country's reference collection, and grown in the trials for DUS. In practice, to include this many varieties in a replicated field trial is logistically and financially impossible for any MS. However, it is desirable that in order to maintain the strength of protection offered by plant breeders' rights (PBR), the principle of comparing new varieties with those of common knowledge should be upheld, and that variety reference collections should be as comprehensive as possible. Clearly, some means of "managing" reference collections is thus highly desirable. Attention has focussed on pre-selection methods, comparing candidate varieties with the reference collection prior to sowing the field trial, in order to on the one hand reduce the number of varieties that need to be grown whilst on the other, maintain the quality of PBR.

One means of such management or pre-selection would be to use molecular markers (DNA profiling) to compare new varieties with those already tested, eliminating those which are sufficiently distant and do not need to be compared in a field trial (according to pre-defined criteria) and then only growing the most similar varieties for detailed morphological DUS assessment. This is the approach examined in this project, which, if successful, may provide potential ways of improving the cost-effectiveness of WOSR DUS testing across the EU (and ultimately more widely), addressing the genotype x environment issue, and enabling increasing work-loads to be achieved within existing resources.

2. Objectives addressed

The overall objective of this project is to examine the potential uses of molecular markers (specifically DNA microsatellites, or simple sequence repeats, SSRs) for the management of variety reference collections in oilseed rape DUS testing. This would be an "Option 2"* approach as previously defined by the BMT, and hence would be acceptable only if it could be demonstrated that there is a relationship or association between the variety "distances" as calculated using molecular markers ("genetic distance"), and those calculated based on the phenotypic markers currently used in DUS testing ("phenotypic distance").

3. Project Outline

The general structure of the project is as follows:

- Selection of molecular markers
- Selection of variety set
- Molecular analysis of variety set
- Compilation of phenotypic data for variety set
- Analysis of data – comparisons of variety distance estimates

4. Progress to Date

4.1 Standardizing Conditions for the Use of an Agreed Set of SSRs.

DNA samples from 10 WOSR varieties were prepared by NIAB and circulated to GEVES and DIAS, along with the primer sequences of 29 SSRs (derived from previous work undertaken by NIAB and GEVES) and a draft analytical protocol. Following preliminary discussions, seed samples of a further 40 varieties were distributed by NIAB for analysis. The samples were analyzed using DNA extracted from thirty seeds per variety using the same markers as before, with an agreed protocol. Comparison of the results from the three laboratories showed that it would be desirable to use as standardized an approach to the genotyping as possible, e.g. use DNA extraction kits to ensure that DNA of comparable quality is used in all laboratories, standardize the PCR protocol in terms of the primer labeling strategy used and the source of Taq polymerase, and use the same analytical platform (preferably capillary-based). Following this exercise, a set of 21 SSR markers were agreed for use in the next phase of the project, along with an agreed analytical protocol and scoring strategy.

4.2 Selection of variety set and analysis of WOSR varieties from different EU MS using the agreed SSRs.

It was initially agreed that the project should analyze only those varieties from the participating countries which were lines (i.e. no hybrids) and fertile (no male-sterile lines). Using these criteria, each partner produced a list of the relevant varieties from their country, and sent these lists to NIAB, where the lists were collated, and a set of 410 varieties compiled. The partners then supplied NIAB with seed samples of the appropriate varieties from their collections, which were coded and the appropriate samples re-distributed to the laboratories undertaking the genotyping work.

* Option 2: Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics (see documents TC/38/14-CAJ/45/5 and TC/38/14 Add-CAJ/45/5 Add.)

The total list of varieties was divided between laboratories - 190 varieties to NIAB, 190 to GEVES and 70 to DIAS. In addition to these, 5 coded samples from the original set of 40 were included, and of the 190 sent to NIAB and GEVES, 40 of these varieties were common to both, for quality control purposes. The varieties were analyzed by each laboratory using the agreed set of SSRs. All laboratories used a capillary based platform for this phase.

4.3 Compilation of phenotypic data from the variety set

Each of the partners provided the phenotypic data available for any of the 410 varieties from the selected variety set. Since it was thought that using only UPOV Notes may result in "clumped data" and consequently rather crude phenotypic distance estimates, it was agreed that phenotypic data would be provided in the form of UPOV Notes for each characteristic listed in the CPVO Technical Protocol. In addition data was collated as variety means for each appropriate continuously assessed characteristic from the years 2003, 2004 and 2005 for which data were available.

4.4 Preliminary distance analyses

Using the genotypic and phenotypic data available at the time (which was incomplete), a preliminary analysis of distance estimations was carried out. The objective was to identify variety pairs to grow in a field trial to be sown in Autumn 2006. These would be varieties which were, for instance, similar in phenotypic distance but easily separable (dis-similar) in terms of genetic distance. The approaches used in this analysis and the results will be discussed in more detail in the presentation.

4.5 Variety pairs sown in field trials

Those variety pairs of interest (i.e. close in morphological distance, but distant using SSR data) for each partner were identified from the preliminary analysis. Seed of each partner's interesting variety pairs was exchanged, so that the pairs were replicated in all countries. The pairs were then sown in side-by-side plots in field trials in Denmark, France, Germany and the United Kingdom for visual examination. All of the characteristics included in the CPVO Protocol will be recorded in the coming season

5. Next Activities

(i) Definitive analysis of complete molecular marker and phenotypic datasets to determine genetic and phenotypic distances, using a range of different statistical approaches
(ii) Potential application of SSR data in the GAÏA software.
(iii) Consider the design of a database of SSR profiles for potential use in DUS testing with the EU.

The presentation will consider in detail the available data and the preliminary analyses and their outcome, and then outline the future activities within the project.

Acknowledgements

CPVO for co-funding
DEFRA for co-funding for the UK

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