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AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR OILSEED RAPE

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CONTRIBUTION PAPER

Document prepared by experts from the United Kingdom

Contribution paper for the *Ad hoc* Oilseed Rape Subgroup on Molecular Techniques

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Introduction

My contribution is arranged according to the work program indicated in Annex II to Circular 3036. I will also make reference to the corresponding Issue Paper BMT/6/14. My contribution will be based on review of relevant publications and my expertise rather than a presentation of new results. I do not attempt to address all parts of the program. My particular expertise is Statistics and I have experience of both DUS tests and molecular techniques.

1. Molecular methods available and suitability for use

a) **Capacity for discriminating varieties**

In answer to the question in paragraph 24 of BMT/6/14, I would say that it is necessary that molecular markers should be usefully polymorphic with the collection of existing protected varieties. Ideally, the markers should be usefully polymorphic across the genome. This may be mean that individual markers should be highly polymorphic, or that an interval of genome is represented by a number of markers. In addition, it seems highly desirable that the markers are evenly distributed or that they are mapped. When markers are not selected by their location on the genome, they can often form clusters of tightly linked loci (e.g. for *brassica oleracea*: Sebastian *et al* 2000) rather than being evenly dispersed. This could have a detrimental effect on estimation of genetic distance. Mapping information could be included in the distance calculations, thus increasing the precision (BMT/6/10).

Studies reported in BMT/5/5 and BMT/6/9 show the potential of molecular markers to discriminate between varieties and identify off-types.

d) – f) **Consistency and reproducibility**

I address a question in paragraph 5 of BMT/6/14 "*To what extent is reproducibility considered necessary for the purpose of DUS examination or the management of reference collections*?" If a variety were not distinct on the basis of morphological characters, then it is possible that a distinctness decision based on molecular markers would be based on only a few markers. Similarly for uniformity, it may be only a few markers that identify off-types. Therefore a high degree of reproducibility would be needed to ensure that another laboratory would make the same decisions using on the same marker set.

2 (a) DUS issues – distinctness

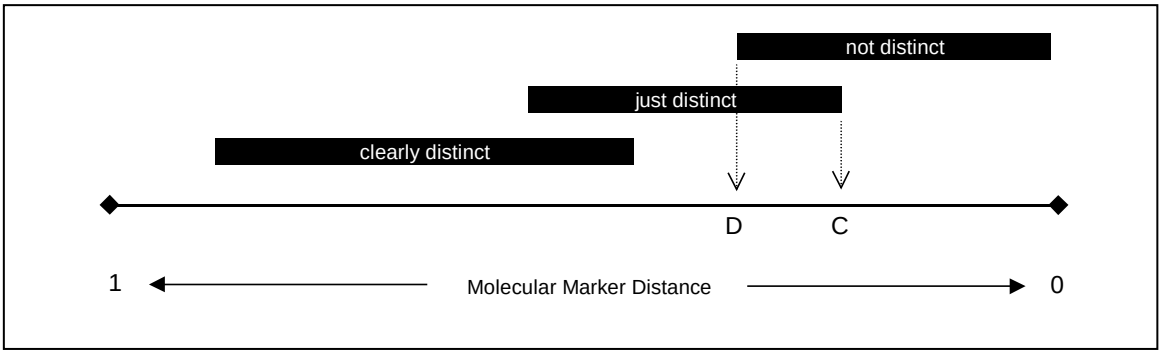
B-1 Use of gene specific markers linked to traditional characteristics

In answer to the question in paragraph 9 of BMT/6/14, if such gene specific markers are to be used to assess distinctness, replacing the corresponding traditional characteristic, then it seems vital to ascertain that the markers can indicate presence or absence of the characteristic with 100% success in the reference collection.

B-2 Use of DNA profiles regardless of their linkage with traditional characteristics

If the markers were to be treated as traditional characteristics, then I would agree with the points made in paragraph 13 of BMT/6/14. In particular, it would be useful to see further studies relating the variability between and within oilseed rape varieties, and as paragraph 14 states, this would be useful information anyway to ascertain the discriminatory power of molecular techniques. Indeed it would be interesting to compare the within-variety variability with the threshold constructed using the concepts indicated in paragraphs 16 and 17.

Addressing the considerations outlined in paragraph 18 of BMT/6/14, I do think the conceptual diagram is useful to illustrate the choices of threshold options, particularly the part on page 6. It may benefit from some simplification. Essentially we are saying that pairs of varieties can be grouped into 3 categories: pairs that are clearly distinct using traditional characteristics; varieties that are only just distinct using traditional characteristics (perhaps differing in only one character); and varieties that are not distinct using traditional characteristics. If we were to map these groups onto a line representing molecular marker distance, it might look like this:



If molecular markers were to be used alongside the traditional characters to establish distinctness, it could be argued that, to justify their use, molecular markers should allow additional discrimination between varieties. This would indicate a preference for position C over B as a threshold for distinctness. On the other hand, if markers were to be used instead of the traditional characteristic, the choice would not be so clear.

I would agree that more data would be needed to carry out the calibration as indicated in paragraph 16 of BMT/6/14, particularly for varieties that are non-distinct using traditional characteristics.

2 (b) DUS issues – uniformity

Addressing the questions put in paragraph 20 of BMT6/14, the assessment of uniformity by number of off-types needs two steps. First off-types should be identified. It seems sensible to identify off-types as individuals that are distinct from the majority of individuals representing the variety. The criteria for distinctness could be the same as for new varieties. After off-types have been identified, the standard tolerance levels (as per the guidelines) could be applied. The identification of off-types is the more difficult part of the process – data would be required (in addition to that supplied in BMT/6/9) to verify that the use of the distinctness criteria leads to similar identification of off-types as for traditional characteristics. It should be borne in mind that assessment of uniformity by off-type may require fingerprinting of a large number of plants.

I agree with the strategy identified in paragraph 21 for cross-pollinated varieties and for where the simple off-type system is not suitable. Data would be required to compare uniformity observed in traditional characteristics and uniformity observed by molecular data. However, if the reference collection is reasonably large, and assessment of uniformity is by comparison with these varieties, this guards against a great change in the criteria. It should be noted again that assessment of uniformity by molecular data would mean the fingerprinting of a large number of plants per variety.

2 (c) DUS issues – stability

No comment.

2 (d) DUS issues – influence of different methods on levels of DUS

The choice of molecular marker set was addressed in section 1 (a) above. The choice of genetic distance method is partly determined by the type of markers to be used and whether a map is available. BMT/5/5 includes a useful discussion on the choice of distance method for AFLP markers with oilseed rape. BMT/6/10 shows how the use of map information may improve the precision of genetic distance estimation.

3 Relationship to phenotype

No comment

4 Potential applications

a) Establishment of DUS (or supporting evidence) and b) Management of reference collections.

It seems that different levels of association between molecular marker distance and morphological distances are required for establishment of DUS using markers and use of markers to screen the reference collection for similar varieties prior to DUS testing. For the latter, the molecular data should help to eliminate reference varieties that are morphologically dissimilar to candidate varieties and/or to select reference varieties that are morphologically similar to candidate varieties (paragraph 29 BMT6/14). However, for establishment of DUS, there seems advantage in having markers that can identify as distinct varieties that are not morphologically distinct, at least if markers are to be used alongside traditional characteristics, rather than replacing their use. Data is required

(paragraph 18 BMT/6/14) for oilseed rape to establish the relationship between marker distances and morphological distances for both morphologically distinct and non-distinct varieties.

b) Assessment of Essential Derivation

This appears to have similarities with paternity testing and thus probabilistic methods may be of use.

5 Possible impacts

No comment.

5 Possible impacts of the introduction and unsolved problems

No comment.

Reference

Sebastian RL, Howell EC, King GJ, Marshall DF and Kearsey MJ (2000)
An integrated AFLP and RFLP Brassica oleracea linkage map from two morphologically distinct doubled-haploid mapping populations TAG 100:75-81

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