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UNIFORMITY ISSUE: OILSEED TURNIP RAPE

Document prepared by experts from the United Kingdom

1. INTRODUCTION.

This submission addresses the work program (Annex II to Circular U 3036) and the corresponding Issue Paper (BMT/6/14). It will attempt to address the "Considerations for Participants" included in Section B-2-2 of the Issue Paper (Uniformity), by relating them to our practical experience in the Distinctness, Uniformity and Stability (DUS) testing of Oilseed Turnip Rape (*Brassica rapa* L. var. *silvestris* (Lam.) Briggs)

Since 1990, the Herbage and Vegetable Section of the Scottish Agricultural Science Agency (SASA) has been undertaking DUS tests in Turnip Rape for UK National Lists and PBR.

Spring Oilseed Turnip Rape is earlier maturing than the much more widely grown Spring Oilseed Swede Rape (*Brassica napus* L.(PARTIM), (often simply called Spring Oilseed Rape). It is suited to the shorter growing seasons but longer summer day-length of the colder climates of Scandinavia, Canada and Scotland. A crucial difference between the two forms is that Turnip Rape is primarily a self-incompatible species, whilst Swede Rape is largely self-pollinating (though the degree of outcrossing in the field due to wind and insects can be as much as 36% (Downey and Rimmer, 1993))

Since 1990, at SASA, 53 candidates have been submitted for UK DUS tests. Sixteen of these were rejected. Five candidates (9% of the total) failed the test on Distinctness criteria and eleven (21%) failed on Uniformity. Of the Uniformity failures, ten (19% of the total) failed due to lack of uniformity of leaf type, namely strap-leafed off-types in a lobed variety (see Fig. 1).

Fig. 1. Photograph illustrating lobed (left) and strap-leafed (right) forms of

If a molecular based early warning system existed for this one category of off-type, it might save a significant amount of the time and cost currently incurred in the registration testing of Turnip Rape varieties.

2. STRAP-LEAVED OFF-TYPES IN LOBED VARIETIES.

The only reference in the literature to the inheritance of leaf shape in *Brassica rapa* can be found in Klein Geltink (1983) and concludes that inheritance of leaf shape is simple, with strap-leaf monogenetically dominant over lobed-leaf. No subsequent studies have challenged this view.

3. UNIFORMITY STANDARDS IN TURNIP RAPE.

UPOV guidelines for the testing of Homogeneity for new varieties of plants were originally set out in TG1/2(1979) and are at present undergoing revision in a number of documents (see, for example, TC/34/5, TC35/13). For vegatatively propagated or self-pollinated varieties a percentage population standard is applied. For cross-pollinating varieties relative tolerance limits for off-type number are used, and these are constructed by comparison with those of "comparable" varieties. Uniformity standards in Turnip Rape differ from those in Swede Rape because of the greater degree of cross-pollination present in the former. (However, it has been our experience in Turnip Rape, where the number of "comparable" varieties is small and their commercial life is short, a fixed Uniformity standard has been more practical. This is because one uniform variety may unduly reduce the relative tolerance limits).

The picture is further complicated by the fact that there exist a number of distinct breeding methods for new Turnip Rape varieties: firstly, the conventional practice of mass selection; secondly hybridisation and thirdly development of synthetic varieties derived from crossing parent populations or lines. SASA has applied a different standard for Uniformity for each of these breeding types (Campbell, 1999).

To reliably determine the number of off-types in Spring Turnip Rape, Uniformity trials at SASA are grown both in the glasshouse and in the field. The number of clear off-types per candidate is recorded over two years. (A separate glasshouse test is preferred for the evaluation of leaf type uniformity, because insect damage to the meristems at cotyledon stage in the field can mimic strap-leafed off-types). Other uniformity evaluations are done in the field.

4. B-2-2. 20 ASKS:

- ✓ *Can the standard tolerance level of off-types (determined in UPOV Test Guidelines or document TC/34/5) be applied?*

In the case of Turnip Rape; if a molecular test of the present band/absent band type were developed and it was 100% correlated with the strap-leafed off-type, there would seem no reason for adjustment to the cross-pollinating relative tolerance limits which currently apply. Such a test might be possible in Turnip Rape due to the relative importance of one form of off-type and its relatively simple genetic background. However for a test, less than 100% correlated, a higher, statistically adjusted limit would seem to be indicated. In either case a large number of plants (or preferably seeds) would need to be tested. At the moment, in Turnip Rape, the number of plants used in the glasshouse to estimate Uniformity is approximately 400 in each of the two years of test. With simple DNA extraction available (e.g. Sigma's REDExtract-N-amp™ PCR kit (http://www.sigma.com/)) and automation, it is now possible to test large numbers of individual seeds. However with such large numbers of

PCR reactions involved, and the cross-pollinated Uniformity standards requiring comparison with other varieties (see above), the task becomes a formidable one - and only tenable if it were to replace the glasshouse uniformity test, not just complement it.

✓ *How can uniformity (off-types) be judged by molecular information?*

A range of molecular techniques will be necessary to evaluate Uniformity, depending upon the genetic make-up of the candidate variety, its mode of reproduction and breeding history. It is also crucially important to understand whether continuous or discontinuous uniformity is being examined. The role of bioinformatics should not be ignored in the quest for markers for the particular traits involved in common off-types. It is possible to search genomic databases (although with little success at present) such as The Nottingham Arabidopsis Stock Centre (NASC) (http://arabidopsis.org.uk/) for genetically characterised phenotypic traits, which might in time lead to the identification of molecular markers in related species. As the amount of data on these databases increases, the prospects for a short cut to these markers must improve.

DUS testers at SASA are generally wary of those molecular techniques (e.g. RAPD, AFLP, SSR) where markers are not tied to a morphological trait, in both Distinctness and Uniformity testing. Reasons for this wariness have been well documented elsewhere. For example, the discussion on the reduction of minimum distances between cultivars using molecular techniques only, and the problem of maintaining and replicating the variety true to its molecular profile.

✓ *Can the same results be achieved for other species and varieties by an appropriate set of molecular markers?*

The AFLP method described by Lombard et al (BMT 6/9) successfully identifies off-types in Swede Rape. The pattern of ALFP marker expression in Turnip Rape would presumably resemble the diversity of Variety C in (BMT 6/9) for most, if not all Turnip Rape varieties. The basis for Uniformity estimation in Turnip Rape using AFLP markers would then follow the Uniformity standards set out for cross-pollinating species (see above), namely a comparison with the AFLP pattern heterogeneity of "comparable" varieties. As is noted in (BMT 6/9), this molecular heterogeneity is not fully reflected in morphological heterogeneity – it is questionable whether such a test would be acceptable to the breeding industry.

SASA are keen to embrace any new molecular and biochemical techniques which make DUS testing more effective. We recognise the valuable research undertaken in this area in recent years. We further recognise that DNA profiling already provides an efficient way of checking varietal identity, but there are questions concerning distinctness and uniformity that remain to be answered. Further work is required to establish just how molecular and biochemical techniques may best complement current testing methods, especially in understanding the genetic basis of the differences observed. Furthermore, it is still not clear how a maintainer can maintain varietal purity based in part on molecular criteria.

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Tom Christie
Herbage and Vegetable Section
SASA
82 Craigs Road
Edinburgh EH12 8NJ
SCOTLAND
+44 (0)131 244 8961
Tom.Christie@sasa.gov.uk

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