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REVISION OF DOCUMENT TGP/8: PART II: SELECTED TECHNIQUES
USED IN DUS EXAMINATION, NEW SECTION: EXAMINING DUS IN BULK SAMPLES

Document prepared by the Office of the Union

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EXECUTIVE SUMMARY

1. The purpose of this document is to report on developments concerning guidance on examining DUS in bulk samples for inclusion in a future revision of document TGP/8.
2. The TWV is invited to consider the proposed guidance for examining DUS in bulk samples as presented in the Annex to this document, for inclusion in a future revision of document TGP/8.
3. The structure of this document is as follows:

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4. The following abbreviations are used in this document:

TC:	Technical Committee
TWA:	Technical Working Party for Agricultural Crops
TWC:	Technical Working Party on Automation and Computer Programs
TWF:	Technical Working Party for Fruit Crops
TWO:	Technical Working Party for Ornamental Plants and Forest Trees
TWPs:	Technical Working Parties
TWV:	Technical Working Party for Vegetables

BACKGROUND

5. The background to this matter is provided in document TWV/49/17 "Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining DUS in Bulk Samples". DEVELOPMENTS IN 2015

Technical Working Parties

6. At their session in 2015, the TWV, TWC, TWA, TWF and TWO considered documents TWV/49/17, TWC/33/17, TWA/44/17, TWF/46/17 and TWO/48/17, respectively (see documents TWV/49/32 Rev. "Revised Report", paragraphs 49 to 57, TWC/33/30 "Report", paragraphs 28 to 35, TWA/44/23 "Report", paragraphs 42 to 47, TWF/46/29 Rev. "Revised Report", paragraphs 45 to 50 and TWO/48/26 "Report", paragraphs 38 to 45).

7. The TWV, TWC, TWA, TWF and TWO noted that the TC, at its fifty-first session, had agreed that further information on fulfilling the requirements of a DUS characteristic should be provided in the example of a characteristic examined on the basis of a bulk sample, and in that regard, considered a discussion paper provided by an expert from the Netherlands on uniformity requirements in bulk characteristics, as reproduced in document TWV/49/17, Annex I.

8. The TWV invited the expert from the Netherlands, with support from the European Union, France and Germany, to continue the work done for improving the discussion paper, and to clarify the possible approaches feasible in the framework of DUS examination and in relation to a specific characteristic compared to the version presented to the TWV.

9. The TWV further agreed that characteristics to be examined on the basis of bulk samples should be carefully considered before inclusion in Test Guidelines.

10. The TWV noted that France, at the fifty-first session of the TC, had offered to provide other examples of characteristics based on bulk samples and invited other members to provide examples, particularly for vegetable crops.

11. The TWC agreed that the elements (a) "Control of the characteristic before it is accepted in the relevant guideline"; (d) "Subplots"; (g) "DNA analysis"; and (i) "Plant number" might be further developed as a basis for guidance on the analysis of characteristics examined on the basis of bulk samples.

12. The TWA considered the discussion paper provided by an expert from the Netherlands on uniformity requirements in bulk characteristics as reproduced in document TWV/49/17, Annex I, concluded as follows:

- before a characteristic observed on the basis of a bulk sample was included in Test Guidelines it should be considered whether it would be useful and necessary for DUS examination.
- approaches (a) "Control of the characteristic before it is accepted in the relevant guideline"; (d) "Subplots"; and (i) "Plant number" should be further developed for the analysis of requirements that a characteristic examined on the basis of bulk samples should fulfill before it is used for DUS testing and producing a variety description.
- approach (g) "DNA analysis" was too general and did not provide useful information for the assessment of uniformity in characteristics observed on the basis of bulk samples. The TWA noted that molecular markers could be used as a method of examining DUS characteristics on the basis of the existence of a reliable link between the marker and the characteristic, in which case the assessment on basis of bulk samples would not be necessary.

13. The TWO agreed with the conclusions of the TWA and further agreed that DNA analysis would only be appropriate for the assessment of characteristics that satisfy the criteria for characteristics set out in the General Introduction and where there is verification of the reliability of the link between the marker and the characteristic, as set out in document TGP/15 "Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)".

14. The TWO noted that varieties of ornamental plants were being developed for new purposes, such as chemical content, and agreed that it would be important to continue the analysis of requirements that a characteristic examined on the basis of bulk samples should fulfill before it is used for DUS testing.

15. The TWV, TWC and TWA considered further information provided by an expert from the Netherlands on the example of a bulk characteristic in the Netherlands: Content of Glyceraphanin, as reproduced in document TWV/49/17, Annex II. The TWA agreed that it would be necessary to analyze the data obtained from the assessment of the characteristic in order to understand the conclusions provided.

16. The TWV, TWC and TWA noted that the TC, at its fifty-first session, had agreed to consider further whether the analysis of individual plants to validate characteristics examined on the basis of bulk samples was necessary, and the possible cost implications, and had invited alternative approaches for the examination of uniformity to be proposed.

17. The TWV agreed that characteristics examined on the basis of bulk samples should be assessed on the basis of the number of plants recommended in the Test Guidelines under Chapter 4.1.4.

18. The TWC considered whether characteristics examined on the basis of bulk samples should be assessed on the basis of the number of plants recommended in the Test Guidelines under Chapter 4.1.4. It agreed that this approach would be preferable from a statistical perspective but noted that such an approach was not feasible for the example provided because of the cost of analysis of Glyceraphanin content for individual plants.

19. The TWF considered whether characteristics examined on the basis of bulk samples should be assessed on the basis of the number of plants recommended in the Test Guidelines under Chapter 4.1.4 and noted that in some members visual observations of fruits from vegetatively propagated fruit crops were usually made on 20 fruits and the assessment of characteristics such as acidity, degrees brix and firmness of flesh, which resulted in destruction of the plant sample, was made on 10 fruits.

20. The TWF agreed that sampling for the assessment of characteristics that resulted in destruction of the plant sample was usually made by harvesting typical fruits from the same part of each tree (same stage of development, middle part of tree) and mixing them together. The appropriate number of fruits (10 or 20) would then be randomly selected for the assessment of each of the characteristics.

DEVELOPMENTS IN 2016

Technical Committee

21. The TC, at its fifty-second session, held in Geneva from March 14 to 16, 2016, considered document TC/52/18 "Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining DUS in Bulk Samples" (see document TC/52/29 Rev. "Revised Report", paragraphs 111 to 113).

22. The TC agreed that the Netherlands should be invited to develop guidance, with the inclusion of examples, for examining DUS in bulk samples for inclusion in a future revision of document TGP/8, on the following basis:

- (a) the characteristic should fulfill the requirements of a characteristic, as set out in the "General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of new Varieties of Plants" (see document TG/1/3, Section 4.2.1);
- (b) there should be knowledge of the genetic control of the characteristic;
- (c) the suitability of the characteristic should be validated through an initial assessment of uniformity on individual plants;
- (d) information on plant-by-plant variation and differences between growing cycles should be provided (data from routine measurement of the characteristic from different years);
- (e) a full description of the method of assessment should be provided;
- (f) states of expression should be based on existing variation between varieties considering environmental influence.

23. The TC agreed that the draft guidance should be considered by the TWPs, at their sessions in 2016.
24. The guidance developed by the Netherlands is reproduced in the Annex to this document.

25. The TWV is invited to consider the proposed guidance for examining DUS in bulk samples as presented in the Annex to this document, for inclusion in a future revision of document TGP/8.

[Annex follows]

STATUS ON UNIFORMITY REQUIREMENTS IN BULK CHARACTERISTICS

Document prepared by an expert from the Netherlands

1. From the discussions in the TWPs in 2015 and in the TC it is clear that there is not much room to deviate from the standard way to observe characteristics in order to establish if the Uniformity requirements are fulfilled.

2. From the options that were proposed in 2015 (see Annex I in documents TWA/44/17, TWC/33/17, TWF/46/17, TWO/48/17 and TWV/49/17) only three approaches (a, d and i) were deemed acceptable by the members of all TWP's. Unfortunately no data were available to further study the pros and cons of these approaches. Therefore it is proposed to accept at this moment only approach a. In future other approaches can be re-discussed.

3. Acceptable approach:

(2015, a) Control of the characteristic before it is accepted in the relevant guideline.

Before a new characteristic is accepted as a bulk characteristic within a UPOV Test Guidelines, the uniformity is checked for a significant number of varieties using a plant by plant method for the required number of plants in the relevant Test Guidelines. In this way it is observed that the characteristic in itself is suitable as a UPOV characteristic on the basis that the uniformity may be checked.

4. Approaches that may be acceptable in future, if data are available and the method is discussed and accepted in the relevant Technical Working Party:

(2015, d) Subplots.

Making use of subplots in order to indicate the uniformity of the characteristic. Only one observation per plot, but there are more subplots in the trial. An example is dry matter content in Onion. There are three subsamples in the trial. It is possible to work with 3 subsamples for an indication of uniformity. (see: TGP/8.6).

(2015, i) Plant number.

Use a different number of plants for this characteristic to be tested in the guideline that is in congruence with the nature of the characteristic. For example: in a certain guideline it is mentioned that 60 plants have to be judged for uniformity. If the characteristic involved is not suitable for judgment of 60 plants, one can propose a lower number of plants for the relevant characteristic for example 5 plants.

5. Item for Re-discussion

In line with the rapid development of DNA techniques, an approach where the uniformity of an application is judged by judging the DNA pattern for the required number of plants in the relevant guideline is well feasible. Therefore it is proposed to re-consider this option as a way to establish the suitability of bulk samples in a characteristic.

[End of Annex and of document]