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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL COMMITTEE

Fifty-Second Session
Geneva, March 14 to 16, 2016

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 TC is invited to:

(a) consider the discussion paper provided by an expert from the Netherlands on uniformity requirements in bulk characteristics and information provided on the example of a bulk characteristic in the Netherlands, as reproduced Annexes I and II to this document;

(b) consider the comments by the TWPs at their sessions in 2015; and

(c) consider the proposal for the development of guidance on examining DUS in bulk samples for inclusion in a future revision of document TGP/8, as presented in paragraph 22.

3. The structure of this document is as follows:

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 TC/51/18 “Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining DUS in Bulk Samples”.

DEVELOPMENTS IN 2015

Technical Committee

6. The TC, at its fifty-first session, held in Geneva, from March 23 to 25, 2015 (see document TC/51/39 “Report”, paragraphs 140 to 145):

- 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;
- 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 invited the TWPs to propose alternative approaches for the examination of uniformity;
- agreed to consider further 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;
- agreed that the determination of states of expression should be based on existing variation between varieties and considering environmental influence; and
- welcomed the offer of France to provide other examples of characteristics based on bulk samples and invited other members to provide examples.

Technical Working Parties

7. 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).

8. 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 Annex I to this document.

9. 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.

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

11. 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.

12. 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.

13. The TWA considered the discussion paper provided by an expert from the Netherlands on uniformity requirements in bulk characteristics as reproduced in Annex I to this document and 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.

14. 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)”.

15. 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.

16. 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 Glycoraphanin, as reproduced in Annex II to this document. 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.

17. 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.

18. 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.

19. 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 Glycoraphanin content for individual plants.

20. 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.

21. 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.

PROPOSAL

22. On the basis of the comments by the TC and TWPs, it is proposed that the following aspects be considered as a basis for the development of guidance on examining DUS in bulk samples for inclusion in a future revision of document TGP/8:

(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 method of assessment should be provided;

(f) states of expression should be based on existing variation between varieties considering environmental influence

23. The TC is invited to:

(a) consider the discussion paper provided by an expert from the Netherlands on uniformity requirements in bulk characteristics and information provided on the example of a bulk characteristic in the Netherlands, as reproduced Annexes I and II to this document;

(b) consider the comments by the TWPs at their sessions in 2015; and

(c) consider the proposal for the development of guidance on examining DUS in bulk samples for inclusion in a future revision of document TGP/8, as presented in paragraph 22.

[Annexes follow]

ANNEX I

DISCUSSION PAPER ON UNIFORMITY REQUIREMENTS IN BULK CHARACTERISTICS

Document prepared by an expert from the Netherlands

1. There are different approaches to show that the uniformity requirements can be fulfilled in a bulk characteristic. In this paper we discuss the possible solutions to test for uniformity in the case of a bulk characteristic with only one sample per plot. In case of more samples per plot see: TGP 8 (Trial design) and TGP/10 (uniformity requirements).

2. There are different approaches to check uniformity that are not discussed in the current TGP´s. In order to discuss the suitability for UPOV the possible approaches are listed here. The discussions within UPOV have two purposes. The first purpose is to make the list of possible approaches exhaustive. The second purpose is to check the relevance of each approach. Further elaboration is required in future of those approaches considered relevant.

3. The possible approaches are listed here:

(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 guideline, 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 guideline. 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.

(b) Check if uniformity must be tested based on type of characteristic and crop.

The need to check on uniformity depends heavily on the type of characteristic and the crop. In a variety that is vegetatively reproduced one can rely on other characteristics for the control of uniformity on the basis of the genetic uniformity of the variety based on the type of propagation. If there are one or more characteristics related to the bulk characteristic, it is possible to rely on the uniformity of the other characteristics. In bean there are 11 seed characteristics. 1000 kernel (grain) weight in beans is a bulk characteristic. There are 10 other characteristics in the seed in bean that are tested for uniformity.

(c) Random Check.

Random check of a characteristic used as a bulk characteristic. Sugar content in Industrial Chicory is a bulk characteristic because of the costs. It is possible to check randomly varieties for the uniformity on a plant by plant basis.

(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).

(e) Other way of checking uniformity: Image analysis.

Use of image analysis. 1000 kernel (grain) weight in beans. It is possible to use image analysis to check the uniformity of this characteristic. In a picture one can observe or calculate the grains with a deviating perimeter. In this way the same characteristic is observed in a different way.

Image analysis may also be used to check uniformity in a general way for the variety as a whole. In that way the conclusion on the basis of image analysis replaces the uniformity check on other characteristics.

(f) Only important characteristics must be checked for uniformity.

A short list of characteristics in a crop to be tested for uniformity. Think about the crop concerned. Which characteristics are important within this crop. Choose several characteristics for the check on uniformity in the relevant UPOV Working Party.

(g) DNA analysis.

Use of DNA analysis. Instead of the uniformity check in the bulk characteristic, an additional test for general uniformity based on a DNA marker set may be used. This is a source of information that delivers an additional test on uniformity. In some cases this will be cheaper than a plant by plant measurement of the characteristic concerned.

As with image analyses the whole check on uniformity of the variety may be replaced by a DNA test.

(h) Cultivation.

Choose another way of growing the variety to check the uniformity. If the normal trial set up is in a row, make use of an addition trial set up plant by plant to check the uniformity of the variety as a whole.

(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.

[Annex II follows]

ANNEX II

AN EXAMPLE OF A BULK CHARACTERISTIC IN THE NETHERLANDS: CONTENT OF GLYCORAPHANIN

1) Consideration if the characteristic is suitable as a characteristic for DUS testing
2) Description of the characteristic
3) Method of detection

1) Consideration if the characteristic is suitable as a characteristic for DUS testing

Selection of Characteristics is mentioned in the technical guidance of the UPOV: TG/1/3 page 9

In this chapter we consider whether the characteristic Content of Glycoraphanin in broccoli does fulfil the requirements of a characteristic in the sense of UPOV.

4.2 Selection of Characteristics

4.2.1 The basic requirements that a characteristic should fulfill before it is used for DUS testing or producing a variety description are that its expression:

(a) results from a given genotype or combination of genotypes
(this requirement is specified in Article I(vi) of the 1991 Act of the UPOV Convention but is a basic requirement in all cases);

The content of Glycoraphanin is stable per variety over the years, but different between varieties.

(b) is sufficiently consistent and repeatable in a particular environment;

The content of Glycoraphanin is stable per variety over three years tested. The results between the contents as stated by the TQ is in congruence with the data recorded in a trial at Naktuinbouw.

(c) exhibits sufficient variation between varieties to be able to establish distinctness;

Very clear.

(d) is capable of precise definition and recognition
(this requirement is specified in Article 6 of the 1961/1972 and 1978 Acts of the UPOV Convention, but is a basic requirement in all cases);

Yes, see method.

(e) allows uniformity requirements to be fulfilled;

At the moment we do not have reasons to doubt the uniformity within this characteristic. As mentioned above the results per variety are stable over the years with only 3 plants per variety. This is an indication that the characteristic is uniform between plants within the variety. Because of the cost aspect we did not yet test the uniformity of 20 plants within several varieties. However technically this is possible to carry out.

(f) allows stability requirements to be fulfilled, meaning that it produces consistent and repeatable results after repeated propagation or, where appropriate, at the end of each cycle of propagation.

Yes. The content of Glycoraphanin was tested over several years.

Conclusion:

In principle all requirements mentioned by UPOV are fulfilled. The uniformity requirement is not fully proven.

2 Description of the characteristic

Type of characteristic
Quantitative characteristic

Characteristic:
Glucoraphanin content

1. Low
2. Medium
3. High

Growth stage
Harvest maturity

Type of observation of characteristic
MG – single Measurement on a Group of plants
(specification in protocol see method)

States of expression (µmol/g DW)
1. 0-15
2. 16-35
3 > 35

Remark
Although genetics play a major role in the glucoraphanin levels in a variety, values can vary due to growing conditions and geographic locations, so the results of measurements should be related to example varieties. The values given in this example are based on repeated trials at Naktuinbouw in the Netherlands.

Example varieties

Low:	Ironman
Medium:	Steel
High:	BRM533934

3 Method of detection (English only)

INB Sample Collection Protocol

Composite samples will be collected for glucosinolate analysis of INB varieties as outlined below.

- Randomly select three market-mature heads from the test plot. Harvested samples must be 5-7 inches in diameter. Trim the sample using the 'square cut' method, such that the height to width ratio of the trimmed head is 1:1. For example a crown that 5 inches in diameter should have the stem trimmed so that the total height is also 5 inches.
- Harvest crowns from the middle of the plot area if possible.
- Harvest should occur early in the morning or late in the afternoon in order to avoid potentially harmful effect of elevated temperatures on plant sample quality.
- Place the three trimmed heads into a plastic bag, accompanied by a single barcode to identify the sample. Label the bags on the outside to include variety name.
- Repeat the selection, harvest, trimming, labeling, and barcode identification process for each plot in the trial.
- All samples should be placed into a cooler with wet or blue ice (no dry ice) to keep them cool until shipped. Samples should be packed in blue ice for overnight shipment.

If possible, all samples should be collected on the same day. Since varieties mature at different rates, it is acceptable to harvest samples that would represent 1st, 2nd or 3rd cuts on the same day and ship as single shipment; as long as all harvested samples represent mature samples that would be commercially acceptable.

Note: In previous years, 3 composite samples (each comprised of 3 heads) were collected and shipped for analysis. Data collect over the previous years on INB varieties have demonstrate that there is little within-plot variation (+/- 12%). Since samples within plots provide very consistent values for glucosinolates, we will collect a single composite sample for each plot going forward. These three head composite samples will generate a single data point.

[End of Annex II and of document]