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TECHNICAL WORKING PARTY ON AUTOMATION AND COMPUTER PROGRAMS**Thirty-Third Session****Natal, Brazil, from June 30 to July 3, 2015**

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 TWC is invited to:
 - (a) consider 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;
 - (b) note that that the TC, at its fifty-first session, 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, consider a discussion paper provided by an expert from the Netherlands on uniformity requirements in bulk characteristics, as reproduced Annex I to this document;
 - (c) note that that the TC, at its fifty-first session, 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 to propose alternative approaches for the examination of uniformity;
 - (d) 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;
 - (e) note that the TC, at its fifty-first session agreed that the determination of states of expression should be based on existing variation between varieties and considering environmental influence; and
 - (f) note the offer of France to provide other examples of characteristics based on bulk samples and invited other members to provide examples.

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4. The following abbreviations are used in this document:

TC:	Technical Committee
TC-EDC:	Enlarged Editorial 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

PURPOSE

5. 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.

BACKGROUND

6. The background to this matter is provided in document TWC/32/17 "Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining DUS in Bulk Samples".

DEVELOPMENTS IN 2014

Technical Working Parties

7. At their sessions in 2014, the TWO, TWF, TWC, TWV and TWA considered documents TWO/47/17, TWF/45/17, TWC/32/17, TWV/48/17 and TWA/43/17 "Revision of document TGP/8: Part II: Selected Techniques used in DUS Examination, New Section 11: Examining DUS in Bulk Samples", respectively.

8. The TWO, TWF and TWV considered the example of a bulk characteristic from the Netherlands and agreed that the scale used should have non-overlapping notes (0-5; ~~5~~6-10; ~~10~~11-15; ...) (see documents TWO/47/28 "Report", paragraph 44, TWF/45/32 "Report", paragraph 35 and TWV/48/43 "Report", paragraph 40, respectively).

9. The TWO noted the information that "[...] 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 [...]". The TWO and the TWA agreed that the usual approach was to confirm uniformity prior to the establishment of stability and that care would be needed on the examination of stability allowing for the establishment of uniformity of a variety for a given characteristic (see documents TWO/47/28, paragraph 45 and TWA/43/27 "Report", paragraph 37).

10. The TWO agreed that examples of other characteristics examined on the basis of bulk samples could be considered for the development of guidance (see documents TWO/47/28, paragraph 46).
11. The TWF and TWV agreed on the development of guidance on the development of characteristics examined on the basis of bulk samples (see documents TWF/45/32, paragraph 36 and TWV/48/43, paragraph 42, respectively).
12. The TWC received a presentation by an expert from the Netherlands on the use of the content of Glycoraphanin in broccoli based on bulk samples, as set out in the Annex to document TWC/32/17 (see document TWC/32/28 "Report", paragraph 34).
13. The TWC agreed that a sufficient number of plants should be used to assess uniformity in bulk samples and the TWC noted that care would be needed to attest stability due to known variation in chemical content in other crops such as oilseed rape (see document TWC/32/28, paragraph 35).
14. The TWA agreed that the example was not supported by sufficient data and agreed with the TWC that the routine measurement of this characteristic in the Netherlands would allow sufficient data set to be generated for further consideration and agreed to invite the Netherlands to provide further information (see document TWA/43/27, paragraph 38 and TWC/32/28, paragraph 36).
15. The TWC agreed that the assessment of uniformity for characteristics based on bulk samples should consider the analysis of individual plants to validate characteristics and noted the possible cost implication of this approach (see document TWC/32/28, paragraph 37).
16. 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 (see document TWV/48/43, paragraph 41).
17. The TWA noted that the states of expression had a fixed scale of values and a remark on variation due to environmental influence. The TWA agreed that the determination of states of expression should be based on existing variation between varieties and considering environmental influence (see document TWA/43/27, paragraph 39).

DEVELOPMENTS IN 2015

Enlarged Editorial Committee

18. The TC-EDC, at its meeting held in Geneva, on January 7 and 8, 2015, considered document TC-EDC/Jan-15/8 "Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining DUS in Bulk Samples".
19. The TC-EDC proposed that further information on fulfilling the requirements of a DUS characteristic be provided in the example of a characteristic examined on the basis of a bulk sample, as presented in the Annex I to this document.

Technical Committee

20. The TC, at its fifty-first session, held in Geneva, from March 23 to 25, 2015, agreed to request the experts from the Netherlands to provide further information on the routine measurement of glycoraphanin content (see document TC/51/18 "Revision of Document TGP/8: PART II: Selected Techniques Used in DUS Examination, New Section: Examining DUS in Bulk Samples", Annex, and TC/51/39 "Report", paragraph 140).
21. The TC, at its fifty-first session, 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 (see documents TC/51/18, Annex and TC/51/39, paragraph 141).

22. The TC, at its fifty-first session, 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. (see document TC/51/39, paragraph 142).

23. The TC, at its fifty-first session, 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 (see document TC/51/39, paragraph 143).

24. The TC, at its fifty-first session, agreed that the determination of states of expression should be based on existing variation between varieties and considering environmental influence (see document TC/51/39, paragraph 144).

25. The TC, at its fifty-first session, welcomed the offer of France to provide other examples of characteristics based on bulk samples and invited other members to provide examples (see document TC/51/39, paragraph 145).

Information provided

26. On May 11, 2015, an expert from the Netherlands provided a discussion paper on uniformity requirements in bulk characteristics, as reproduced Annex I to this document.

27. On June 8, 2015, an expert from the Netherlands provided further information on the example of a bulk characteristic in the Netherlands: Content of Glyceraphanin, as reproduced in Annex II to this document.

Matters for consideration by the TWC

28. In accordance with the request of the TC, the TWC may wish to;

(a) consider 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 Annex II to this document;

(b) note that that the TC, at its fifty-first session, 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, consider a discussion paper provided by an expert from the Netherlands on uniformity requirements in bulk characteristics, as reproduced Annex I to this document;

(c) note that that the TC, at its fifty-first session, 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 to propose alternative approaches for the examination of uniformity;

(d) 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;

(e) note that the TC, at its fifty-first session agreed that the determination of states of expression should be based on existing variation between varieties and considering environmental influence; and

(f) note the offer of France at the TC, at its fifty-first session, to provide other examples of characteristics based on bulk samples and invited other members to provide examples.

29. *The TWC is invited to;*

(a) *consider 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 Annex II to this document;*

(b) note that that the TC, at its fifty-first session, 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, consider a discussion paper provided by an expert from the Netherlands on uniformity requirements in bulk characteristics, as reproduced Annex I to this document;

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(e) note that the TC, at its fifty-first session agreed that the determination of states of expression should be based on existing variation between varieties and considering environmental influence; and

(f) note the offer of France to provide other examples of characteristics based on bulk samples and invited other members to provide examples.

[Annexes follow]

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 additional 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]

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 ($\mu\text{mol/g DW}$)

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

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 ($\pm 12\%$). 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 Annexes and of document]