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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
 Geneva

ENLARGED EDITORIAL COMMITTEE
Geneva, January 7 and 8, 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

Disclaimer: this document does not represent UPOV policies or guidance

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 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

BACKGROUND

3. The background to this matter is provided in document TC/50/24 "Revision of document TGP/8: Part II: Selected Techniques Used in DUS Examination, New Section: Examining DUS in Bulk Samples".

DEVELOPMENTS IN 2014

Technical Committee

4. The TC, at its fiftieth session, held in Geneva, from April 7 to 9, considered document TC/50/24 "Revision of document TGP/8: Part II: Selected Techniques used in DUS Examination, New Section: Examining DUS in Bulk Samples" and invited experts from France and the Netherlands to provide examples of their experience in the development of characteristics based on bulk samples, for seed- and vegetatively propagated varieties, as a basis to develop guidance on the development of characteristics examined on the basis of bulk samples (see document TC/50/36 "Report on the Conclusions", paragraph 53).

Technical Working Parties

5. 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, respectively.

6. 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; ~~56~~-10; ~~1011~~-15; ...) (see documents TWO/47/28 "Report" paragraph 44, TWF/45/32 "Report" paragraph 35 and TWV/48/43 "Report", paragraph 40, respectively)
7. 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 agreed 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 "Report" paragraph 45 and TWA/43/27 "Report" paragraph 37).
8. 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 "Report" paragraph 46).
9. 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 "Report" paragraph 36 and TWV/48/43 "Report", paragraph 42, respectively)
10. The TWC received a presentation by an expert from the Netherlands on the use of the content of Glyceraphanin 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).
11. 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 "Report", paragraph 35).
12. The TWA agreed that the example was not supported by sufficient data (see document TWA/43/27 "Report", paragraph 38).
13. The TWC noted and the TWA agreed 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 TWC/32/28 "Report", paragraph 36 and TWA/43/27 "Report", paragraph 38).
14. 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 "Report", paragraph 37).
15. The TWV agreed that characteristics examined on the basis of bulk samples should be assessed on the basis on the number of plants recommended in the Test Guidelines under chapter 4.1.4. (see document TWV/48/43 "Report" paragraph 41).
16. 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 "Report", paragraph 39).
17. *The TC-EDC is invited to note the information in this document to be presented to the TC and propose any improvements to the document in that regard.*

[Annex follows]

ANNEX

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 if 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 very well 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

- 3. Low
- 5. Medium
- 7. 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. 0-5
- 2. 5-10
- 3. 10-15
- 4. 15-20
- 5. 20-25
- 6. 25-30
- 7. 30-35
- 8. 35-40
- 9 > 40

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

Note

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 (+/- 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 and of document]