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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

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## TECHNICAL WORKING PARTY ON AUTOMATION AND COMPUTER PROGRAMS

### Thirty-First Session Seoul, Republic of Korea, June 4 to 7, 2013

#### SUMMARY OF ASSESSING UNIFORMITY BY OFF-TYPES ON THE BASIS OF MORE THAN ONE SAMPLE OR SUB SAMPLES

*Document prepared by the Office of the Union*

1. The purpose of this document is to report on developments concerning a summary of approaches used by members of the Union in assessing uniformity by off-types on the basis of more than one sample or sub-sample.

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                         |

3. The structure of this document is as follows:

|  |   |
|--|---|
| I. BACKGROUND .....  | 2 |
| II. DEVELOPMENTS IN 2012 .....                                       | 2 |
| Technical Working Party for Agricultural Crops .....                 | 2 |
| Technical Working Party for Vegetables .....                         | 2 |
| Technical Working Party on Automation and Computer Programs .....    | 3 |
| Technical Working Party on Fruit Crops .....                         | 3 |
| Technical Working Party for Ornamental Plants and Forest Trees ..... | 3 |
| III. DEVELOPMENTS IN 2013 .....                                      | 4 |
| Technical Committee .....  | 4 |
| IV. SUMMARY OF APPROACHES .....                                      | 4 |

|            |   |
|------------|---|
| ANNEX I:   | Situation A: Two Growing Cycles in a single location                                  |
| ANNEX II:  | Situation B: Two Growing Locations in the same year                                   |
| ANNEX III: | Situation C: More than one test/trial for a characteristics in the same growing cycle |
| ANNEX IV:  | Situation D: Assessing sub-samples within a single test/trial                         |
| ANNEX V:   | Use of a stepwise approach in the off-type procedure within the same growing cycle    |

## I. BACKGROUND

4. The complete background to this matter is provided in document TWC/30/9 Rev. "Assessing Uniformity by Off-Types on the Basis of More than One Sample or Sub-Sample".

## II. DEVELOPMENTS IN 2012

### Technical Working Party for Agricultural Crops

5. The TWA, at its forty-first session, held in Angers, France, from May 21 to 25, 2012 considered document TWA/41/9 "Assessing uniformity by off-types on the basis of more than one sample or sub-sample" (see document TWA/41/34 "Report", paragraph 46).

6. The TWA, at its forty-first session, agreed that clarification should be provided for Situations A and B if the approach combining the results from two growing cycles was considered to correspond to the requirement for "independent" growing cycles (see document TWA/41/34 "Report", paragraph 47).

7. The TWA, at its forty-first session, noted the explanation from the expert from the Czech Republic that the Apple example should be deleted, because the same approach was used as for other crops (see document TWA/41/34 "Report", paragraph 48).

8. On the basis of information provided at the meeting on "Situation B: Two growing locations in the same year, Approach: Third growing cycle in case of inconsistent results", the TWA agreed to revise the text to read as follows:

"[...] If the variety is within the uniformity standard in one growing location but is not within the uniformity standard in the other growing location, then:

Alternative (a) the trial is repeated at both locations for a second year;

Alternative (b) the trial is repeated at the Leading station (location) (European Union)"

(see document TWA/41/34 "Report", paragraph 49).

9. In the case of "Situation D: Assessing sub-samples within a single test/trial, Approach: Sub-sample as first step of assessment", the TWA agreed that the explanation should be generalized (i.e. no reference to 0 off-types in the subsample) and should provide an explanation of the statistical basis for the approach. The TWA also agreed that the statistical experts from France and Germany should be invited to explain the statistical basis for the acceptable number of off-types in the subsample of 20 plants used in the context of a sample size of 100 plants (see document TWA/41/34 "Report", paragraph 50).

10. The TWA, at its forty-first session, noted that the TWC would be invited to provide guidance on the possible consequences of different approaches (see document TWA/41/34 "Report", paragraph 51).

### Technical Working Party for Vegetables

11. The TWV, at its forty-sixth session, held near the city of Venlo, Netherlands, from June 11 to 15, 2012, considered document TWV/46/9 "Assessing uniformity by off-types on the basis of more than one sample or sub-sample". The changes in document TWV/46/9 from document TWA/41/9 were made, on the basis of the comments made by the TWA, at its forty-first session, as follows:

- deletion of Approach: "Requirement to meet uniformity standard in both growing cycles" (proposed for the Apple example), in section "Summary of approaches" and in Annex I "Situation A: *Two growing cycles in single location*", as set out in paragraph 10 of this document
- revision of the text for Approach: "Third growing cycle in case of inconsistent results" in Annex II "Situation B: *Two growing locations in the same year*," as set out in paragraph 11 of this document

12. The TWV, at its forty-sixth session, noted the different approaches and the similarity between the approaches used in different UPOV members. It agreed to invite the Technical Working Party on Automation and Computer Programs (TWC) to advise whether to use individual or combined results. The experts from

Germany, Italy, France and the Netherlands offered to provide examples and data to the TWC, if needed (see document TWV/46/41 "Report", paragraph 55).

13. The TWV, at its forty-sixth session, agreed that the definition of sample size should be more precise (see document TWV/46/41 "Report", paragraph 56).

#### Technical Working Party on Automation and Computer Programs

14. The TWC, at its thirtieth session, held in Chisinau, Republic of Moldova, from June 26 to 29, 2012, considered document TWC/30/9 "Assessing uniformity by off-types on the basis of more than one sample or sub-sample", with section "Summary of approaches" and annexes, as set out in this document.

15. The TWC, at its thirtieth session, noted the need for further explanation on the situations described, such as the clarification of whether two growing cycles related to the use of the same sample and were carried out in the same year. The TWC agreed that more detailed information and further analysis were needed in order to give guidance on consequences on the use of the different approaches (see document TWC/30/41, "Report", paragraph 83).

16. The TWC, at its thirtieth session, agreed that France, Germany and the Netherlands would present one or more concrete situations in their countries and the statistical basis of their analysis for its next session (see document TWC/30/41, "Report", paragraph 84).

17. In that regard, the Office of the Union has been informed by the experts from France, Germany and the Netherlands that no further information could be prepared for consideration by the TWC at its thirty-first session, to be held in Seoul, Republic of Korea, from June 4 to 7, 2013, and will be prepared for the TWP sessions in 2014.

18. The TWC, at its thirtieth session, agreed that the statistical basis for the acceptable number of off-types in the subsample of 20 plants used in the context of a sample size of 100 plants (situation D) would be assessed by experts from France and Germany (see document TWC/30/41, "Report", paragraph 85).

19. Annex V to this document presents information on the statistical basis for the acceptable number of off-types in the subsample of 20 plants used in the context of a sample size of 100 plants, as prepared by experts from Germany.

#### Technical Working Party on Fruit Crops

20. The TWF, at its forty-third session, held in Beijing, China, from July 30 to August 3, 2012, considered document TWF/43/9 Rev. "Assessing uniformity by off-types on the basis of more than one sample or sub-sample", with section "Summary of approaches" and annexes, as set out in this document.

21. The TWF, at its forty-third session, noted the different approaches and the similarity between the approaches used by different UPOV members. It agreed to propose that the Technical Working Party on Automation and Computer Programs (TWC) be invited to advise whether to use individual or combined results (see document TWF/43/38 "Report", paragraph 51).

22. The TWF, at its forty-third session, requested that the expert from New Zealand would provide a presentation on the testing of uniformity of apple varieties arising from mutations, at the TWF meeting in 2013 (see document TWF/43/38 "Report", paragraph 52).

23. The TWF, at its forty-third session, agreed that the definition of growing cycles should be more precise and that, in the future, a detailed description of the way that the examination was to be conducted should be included under "Matters for future consideration" (see document TWF/43/38 "Report", paragraph 53).

#### Technical Working Party for Ornamental Plants and Forest Trees

24. The TWO, at its forty-fifth session, held in Jeju, Republic of Korea, from August 6 to 10, 2012, considered document TWO/45/9 "Assessing uniformity by off-types on the basis of more than one sample or sub-sample", with section "Summary of approaches" and annexes, as presented in this document. The TWO

noted the different approaches used in different UPOV members (see document TWO/45/37 "Report", paragraph 54).

### III. DEVELOPMENTS IN 2013

#### Technical Committee

25. The TC, at its forty-ninth session held in Geneva from March 18 to 20, 2013, considered document TC/49/14 (see document TC/49/41 "Report on the Conclusions", paragraph 114).

26. The TC noted that the TWC would consider further information on the situations presented in Annex I to IV to document TC/49/14, such as the clarification of whether two growing cycles related to the use of the same sample and were carried out in the same year. The TC noted that the TWC had agreed that more detailed information and further analysis were needed in order to give guidance on consequences on the use of the different approaches. The TWC had further agreed that France, Germany and the Netherlands would present one or more concrete situations in their countries and the statistical basis of their analysis for its next session, and that the statistical basis for the acceptable number of off-types in the sub-sample of 20 plants used in the context of a sample size of 100 plants (situation D) would be assessed by experts from France and Germany (see document TC/49/41 "Report on the Conclusions", paragraph 115).

27. The TC agreed that the approach combining the results from two growing cycles, as set out in Annexes I and II, Situation A and B, was not inconsistent with the requirement for "independent" growing cycles. However, it agreed that care would be needed, for example when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle (see document TC/49/41 "Report on the Conclusions", paragraph 116).

28. The TC noted that an expert from New Zealand would make a presentation on testing of uniformity of Apple varieties arising from mutation at the TWF session in 2013 (see document TC/49/41 "Report on the Conclusions", paragraph 117).

29. Subsequent to the TC meeting, the expert from New Zealand agreed to prepare a document to be presented at all the TWP sessions in 2013 (see document TWC/31/26).

### IV. SUMMARY OF APPROACHES

30. Annexes I to IV to this document, as amended on the basis of the comments made by the TWA, as set out in paragraphs 10 and 11 of this document and considered by the TWV, TWC, TWF and TWO, at their sessions in 2012, summarize different situations when different samples are combined for the overall assessment of uniformity of a variety, as follows:

#### *Annex I: Situation A: Two growing cycles in a single location*

Approach: Third growing cycle in the case of inconsistent results

Approach: Combining the results of two growing cycles

#### *Annex II: Situation B: Two growing locations in the same year*

Approach: Third growing cycle in the case of inconsistent results

Approach: Combining the results of two locations

#### *Annex III: Situation C: More than one test/trial in the same growing cycle*

Approach: Additional growing cycle in the case of inconsistent results

#### *Annex IV: Situation D: Assessing sub-samples within a single test/trial*

Approach: Sub-sample as a first step of assessment

31. The summary in Annexes I to IV only relates to situations where more than one sample, or sub-sample, concern the examination of the same characteristic. In the case of different samples, or

sub-samples (e.g. special test), to examine a different characteristic there is no requirement to combine the results because a variety is required to be uniform for all relevant characteristics.

32. *The TWC is invited to:*

(a) *note that the TWC agreed that more detailed information and further analysis were needed in order to give guidance on consequences on the use of the different approaches presented in Annex I to IV of this document, and that France, Germany and the Netherlands would present one or more concrete situations in their countries and the statistical basis of their analysis for its next session;*

(b) *consider the statistical basis for the acceptable number of off-types in the subsample of 20 plants used in the context of a sample size of 100 plants, as provided in Annex V to this document, and whether the same approach could be used for other sample and subsample sizes;*

(c) *note with regard to the approach combining the results from two growing cycles, as set out in Annexes I and II, Situation A and B, the TC agreed that care would be needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle; and*

(d) *note information on testing of uniformity of Apple varieties arising from mutation in New Zealand is presented in document TWC/31/26.*

[Annexes follow]

SITUATION A: TWO GROWING CYCLES IN A SINGLE LOCATION

**Approach: Third growing cycle in the case of inconsistent results**

(Bulgaria, Chile (Wheat), Czech Republic, European Union (Potato), Germany, Georgia, Italy (Potato), Ireland (Potato) Latvia, New Zealand (Lettuce and Apple mutations)).

A variety is considered uniform if it is within the uniformity standard in both of the two growing cycles.

A variety is considered non-uniform if it fails to meet the uniformity standard in both of the two growing cycles.

If at the end of the two growing cycles the variety is within the uniformity standard in one growing cycle but is not within the uniformity standard in the other growing cycle, then uniformity is assessed in a third growing cycle after consultation with the applicant. If in the third growing cycle the variety is within the uniformity standard, the variety is considered uniform. If in the third growing cycle the variety fails to meet the uniformity standard, the variety is considered non-uniform.

**Approach: Combining the results of two growing cycles**

Republic of Moldova, Chile (Potato), Spain (Cauliflower)).

A variety is considered uniform if it is within the uniformity standard in both of the two growing cycles.

A variety is considered non-uniform if it fails to meet the uniformity standard in both of the two growing cycles.

A variety is considered uniform if the total number of off-types at the end of the two growing cycles does not exceed the number of allowed off-types for the combined sample.

[Annex II follows]

SITUATION B: TWO GROWING LOCATIONS IN THE SAME YEAR

**Approach: Third growing cycle for inconsistent results**

A variety is considered uniform if it is within the uniformity standard in both of the growing locations.

A variety is considered non-uniform if it fails to meet the uniformity standard in both of the growing locations.

If the variety is within the uniformity standard in one growing location but is not within the uniformity standard in the other growing location, then

Alternative (a) the trial is repeated at both locations for a second year;

Alternative (b) the trial is repeated at the Leading station (location)  
(European Union(Cauliflower))

**Approach: Combining the results of two locations**

(France (Cauliflower))

A variety is considered uniform if it is within the uniformity standard in both locations.

A variety is considered non-uniform if it fails to meet the uniformity standard in both locations.

A variety is considered within the uniformity standard if the number of off-type plants or parts of plants does not exceed the allowed number of off-types for the combined sample (two locations).

[Annex III follows]

SITUATION C: MORE THAN ONE TEST/TRIAL FOR A CHARACTERISTIC IN THE SAME GROWING CYCLE

**Approach: Additional growing cycle in the case of inconsistent results**

(Bulgaria, Czech Republic, Germany, Hungary, Italy (Wheat), New Zealand (Wheat) Spain (Wheat)).

A variety is considered to be uniform for a characteristic if it is within the uniformity standard for the characteristic in all tests/trials.

A variety is considered non-uniform if it fails to meet the uniformity standard for the characteristic in all tests/trials.

In the case where a variety is within the uniformity standard for the characteristic in one test/trial (e.g. main trial) and not in another test/trial (e.g. ear-row plot), both tests/trials are repeated in a further growing cycle.

[Annex IV follows]

SITUATION D: ASSESSING SUB-SAMPLES WITHIN A SINGLE TEST/TRIAL

Wheat (Croatia, Czech Republic, Estonia, European Union, Germany, Hungary, Norway, Spain, Sweden)  
Barley (Italy)

**Approach: Use of sub-sample as a first step of assessment**

A variety is considered uniform if no off-types are observed in a sub-sample.

A variety is considered non-uniform if the number of off-types in the sub-sample exceeds the accepted number of off-types for the whole sample.

If the number of off-types is 1 or more, but below the accepted number of off-types for the whole sample, the whole sample is assessed.

[Annex V follows]

## USE OF A STEPWISE APPROACH IN THE OFF-TYPE PROCEDURE WITHIN THE SAME GROWING CYCLE

Last year the TWC agreed that the statistical basis for the acceptable number of off-types in the subsample of 20 plants used in the context of a sample size of 100 plants (situation in the European Union ) would be assessed by experts from France and Germany (see document TWC/30/41, "Report", paragraph 85).

The method of uniformity assessment on the basis of off-types (off-types procedure) was described in document TGP/8 starting on page 86. In paragraph 8.1.7 there are statements regarding to the use of the off-types procedure on more than one single test. The description includes a combined test, a two-stage test and sequential tests.

A combined test is described as follows:

Make a decision after two (or three) years based on the total number of plants examined and the total number of off-types recorded.

A two-stage test is described as follows:

Use the result of the first year to see if the data suggests a clear decision (reject or accept). If the decision is not clear then proceed with the second year and decide after the second year.

A sequential test is a multi-stage test where decision rules can be defined dependently or independently on results of the test.

The situation in Germany and in other countries for example for wheat is as described in document TWC/29/09 "Assessing uniformity by off-types on the basis of more than one sample or sub-sample", Annex I, on page 13.

The situation II is the case which is to discuss. This is an example of a two-step test for the assessment of uniformity observed on a sample size of 100 plants or parts of plants. The population standard is fixed with 1%, the acceptance probability with 95% for each decision.

In a first step 20 plants or parts of plants are observed.

- If there are no off-type plants in 20 plants then the variety does not exceed the number of allowed off-types for this characteristic for this growing cycle
- If there are more than 3 off-type plants then the variety exceeds the number of allowed off-types for this characteristic for this growing cycle.
- If there are 1, 2 or 3 off-type plants then the procedure has to continue by a second step

In a second step further 80 plants or part of plants are observed.

- If there are 3 or less off-type plants in 100 (20 of step 1 + 80 of step 2) plants then the variety does not exceed the number of allowed off-types for this characteristic for this growing cycle
- If there are more than 3 off-type plants in 100 (20 of step 1 + 80 of step 2) plants then the variety exceeds the number of allowed off-types for this characteristic for this growing cycle

The decision rule is defined as follows:

A variety is considered within the uniformity standard in a given growing cycle if the number of off-types in all samples does not exceed the number of allowed off-types in either of the samples.

A variety is considered to be uniform if it is within the uniformity standard in both of two growing cycles. If at the end of the two growing cycles the variety is within the uniformity standard in one growing cycle but not within the uniformity standard in the other growing cycle, then the uniformity standard is assessed in a third growing cycle. If at the end of the third growing cycle the variety is within the uniformity standard, the variety is considered uniform. If at the end of the third growing cycle the variety fails to meet the uniformity standard, the variety is considered non uniform.

So the situation is the following:

For a number of characteristics a so-called stepwise approach can be used in each growing cycle. The steps are the samples (20 plants or 20 + 80 plants).

To compare different tests and decision rules it is useful to compare appropriate type-I and type-II errors. Basic ideas were described by experts from France in document TWC/13/17 "Sequential analysis".

Important to know:

Each decision of uniformity of a variety has always a so-called type-II error (acceptance of null hypothesis).

Each decision of non-uniformity of a variety has a so-called type-I error (rejection of null hypothesis).

For example:

It is assumed that the population standard is 1% and the acceptance probability is 95%. All risks are evaluated on the basis of the binomial distribution. To compute the type-II error the population standard for the non-uniform varieties is assumed as 2% (two times 1%).

If we have 20 plants the number of allowed off-types is normally 1.

If we have 20 plants in the first step of the two-step procedure (see above) it is defined that the number of allowed off-types is zero.

If we have 20 plants in the first step and no off-type plants then the type-II error (beta risk) is 66.8%. This is high but comparable with the case if there are 2 off-types within 100 plants. In this case the type-II error is 67.7%. The number of allowed off-types within 100 plants is 3.

Looking on type-I error we find 1.7% in case of 20 plants and 1 off-type and 1.8% in case of 3 off-types in 100 plants.

A decision on the basis of a sample of 20 plants is only taken if there are no off-types which represent a smaller error than the decision taken on 100 plants.

If we have 20 plants in the first step and more than 3 off-type plants then the type-I error (alpha risk) is nearly zero. This is very small and smaller than in the case if there are more than 3 off-types within 100 plants. In this case the type-I error (alpha risk) is 0.3%.

If we have 20 plants in the first step and 1, 2 or 3 off-type plants then we are looking on the next 80 plants and decide on the basis of 100 plants. In that case the type-I and type-II errors have to be evaluated using special formulas or using the software from France ([http://www.seedtest.org/en/stats\\_tool\\_box\\_content---1--1143.html](http://www.seedtest.org/en/stats_tool_box_content---1--1143.html)).

So it is possible to compare all the other situations.

If we modify the assumption of the population standard for non-uniform varieties we get different results.

The open question is: Do we need a statement regarding to the different types of errors for each decision or do we need a statement over all decisions. This is to be discussed with crop experts.

[End of Annex V and of document]