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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

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## TECHNICAL WORKING PARTY FOR ORNAMENTAL PLANTS AND FOREST TREES

### Forty-Fifth Session

Jeju, Republic of Korea, August 6 to 10, 2012

REVISION OF DOCUMENT TGP/8:  
PART I: DUS TRIAL DESIGN AND DATA ANALYSIS  
New Section: Minimizing the Variation due to Different Observers

*Document prepared by an expert from the Netherlands*

#### BACKGROUND

1. The Technical Committee (TC), at its forty-eighth session, held in Geneva from March 26 to 28, 2012, considered the revision of document TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability" on the basis of document TC/48/19 Rev. The TC noted that new drafts of relevant sections would need to be prepared by April 26, 2012, in order that the sections could be included in the draft to be considered by the Technical Working Parties (TWPs) at their sessions in 2012 (see document TC/48/22 "Report on conclusions" paragraph 49).
2. The TC, at its forty-eighth session, agreed to request the drafter to prepare a new draft of the Section on the basis of the comments made by the TWPs in 2011, as set out in document TC/48/19 Rev., Annex II (see document TC/48/22 "Report on conclusions" paragraph 51).
3. The comments of the TWPs on the proposed text for the New Section - Minimizing the Variation due to Different Observers were as follows:

|         |  |     |
|---------|--|-----|
| General | The TWA noted the information provided in Annex II and recommended to replace the title of the first heading "Control of variation due to different observers" by "Minimizing the variation due to different observers" and to delete "and this procedure should preferably be described in ISO Guidelines" at the end of the paragraph on "Training".   | TWA |
|         | The TWC agreed with the comments made by the TWA at its fortieth session and agreed that Mr. Gerie van der Heijden (Netherlands) and Mr. Adrian Roberts (United Kingdom) should prepare a new document taking into account the information contained in document TWC/25/12 Rev. "Review of Test Design: Checking Levels of Quality (Revised)".   | TWC |
|         | The TWV agreed that the information provided in Annex II, provided valuable information that should be included in document TGP/8. With regard to the proposal of the TWC that a new version of that guidance should be prepared taking into account the information contained in document TWC/25/12 Rev. "Review of Test Design: Checking Levels of Quality (Revised)", it concluded that the volume of information provided in document TWC/25/12 Rev. would detract from the main purpose of the document and suggested that a cross-reference might be made to such information. | TWV |
|         | The TWF considered information in Annex II, and agreed that it provided valuable information that should be included in document TGP/8, however it did not come to an agreement on how the section "Testing the calibration" should be handled. It concluded that a revision should go ahead in order to make it   | TWF |

|                         |   |     |
|-------------------------|---|-----|
|                         | less prescriptive.  |     |
| Training                | The TWA noted the information provided in Annex II and agreed that example varieties illustrating the range of expressions can also be a useful element in the training of experts (see paragraph 2 (Training)).  | TWA |
| Testing the calibration | The TWO agreed that the Section "Testing the calibration" should be revised to reflect the likelihood that inexperienced observers would not be entrusted to make VG observations, whilst inexperienced observers might be entrusted to make MG and MS observations. The TWO agreed that the guidance on different types of training and calibration for DUS experts and for staff that would undertake specified measurements should be reflected in the document. | TWO |

4. The draft of New Section: "Minimizing the Variation due to Different Observers" is provided in the Annex to this document.

[Annex follows]

## ANNEX

## TGP/8/1: PART I: NEW SECTION: MINIMIZING THE VARIATION DUE TO DIFFERENT OBSERVERS

1. Introduction

1.1 Variation in measurements or observations can be caused by many different factors, like the type of crop, type of characteristic, year, location, trial design and management, method and observer. Especially for visually assessed characteristics (QN/VG or QN/VS) differences between observers can be the reason for large variation and potential bias in the observations. An observer might be less well trained, or have a different interpretation of the characteristic. So, if observer A measures variety 1 and observer B variety 2, the difference measured might be caused by differences between observers A and B instead of differences between varieties 1 and 2. Clearly, our main interest lies with the differences between varieties and not with the differences between the observers. It is important to realize that the variation caused by different observers cannot be eliminated, but there are ways to control it.

2. Training

2.2 Training of new observers is essential for consistency and continuity of plant variety observations. Calibration manuals, supervision and guidance by experienced observers as well as the use of example varieties illustrating the range of expressions are useful ways to achieve this.

2.1 UPOV test guidelines try to harmonize the variety description process and describe as clearly as possible the characteristics of a crop and the states of expression. This is the first step in controlling variation and bias. However, the way that a characteristic is observed or measured may vary per location or testing authority. Calibration manuals made by the local testing authority are very useful for the local implementation of the UPOV test guideline. Where needed these crop-specific manuals explain the characteristics to be observed in more detail, and specify when and how they should be observed. Furthermore they may contain pictures and drawings for each characteristic, often for every state of expression of a characteristic. The calibration manual can be used by inexperienced observers but are also useful for more experienced or substitute observers, as a way to recalibrate themselves.

3. Testing the calibration

3.1 After training an observer, the next step could be to test the performance of the observers in a calibration experiment. This is especially useful for inexperienced observers who have to make visual observations (QN/VG characteristics). If making VG observations, they should preferably pass a calibration test prior to making observations in the trial. But also for experienced observers, it is useful to test themselves on a regular basis to verify if they still fulfill the calibration criteria.

3.2 A calibration experiment can be set up and analyzed in different ways. Generally it involves multiple observers, measuring the same set of material and assessing differences between the observers.

3.3 In general, inexperienced observers are less likely to be entrusted to make VG observations but might be entrusted to make MG and MS observations.

4. Testing the calibration for QN/MS characteristics

4.1 For observations made by measurement tools, like rulers (often QN/MS characteristics), the measurement is often made on an interval or ratio scale. In this case, the approach of Bland and Altman (1986) can be used. This approach starts with a plot of the scores for a pair of observers in a scatter plot, and compare it with the line of equality (where  $y=x$ ). This helps the eye gauging the degree of agreement between measurements of the same object. In a next step, the difference per object is taken and a plot is constructed with on the y-axis the difference between the observers and on the x-axis either the index of the object, or the mean value of the object. By further drawing the horizontal lines  $y=0$ ,  $y=\text{mean}(\text{difference})$  and the two lines  $y = \text{mean}(\text{difference}) \pm 2 \times \text{standard deviation}$ , the bias between the observers and any outliers can easily be spotted. Similarly we can also study the difference between the measurement of each observer and the average measurement over all observers. Test methods like the paired t-test can be applied to test for a significant deviation of the observer from another observer or from the mean of the other observers.

4.2 By taking two measurements by each observer of every object, we can look at the differences between these two measurements. If these differences are large in comparison to those for other observers, this observer might have a low repeatability. By counting for each observer the number of moderate and large outliers (e.g. larger than 2 times and 3 times the standard deviation respectively) we can construct a table of observer versus number of outliers, which can be used to decide if the observer fulfills quality assurance limits.

4.3 Other quality checks can be based on the repeatability and reproducibility tests for standard measurement methods as described in ISO 5725-2. Free software is available on the ISTA website to obtain values and graphs for seed laboratory tests according to this ISO standard.

4.4 In many cases of QN/MS, a good and clear instruction usually suffices and variation or bias in measurements between observers is often negligible. If there is reason for doubt, a calibration experiment as described above can help in providing insight in the situation.

## 5. Testing the calibration for QN/VS or QN/VG characteristics

5.1 For the analysis of ordinal data (QN/VS or QN/VG characteristics), the construction of contingency tables between each pair of observers for the different scores is instructive. A test for a structural difference (bias) between two observers can be obtained by using the Wilcoxon Matched-Pairs test (often called Wilcoxon Signed-Ranks test).

5.2 To measure the degree of agreement the Cohen's Kappa ( $\kappa$ ) statistic (Cohen, 1960) is often used. The statistic tries to account for random agreement:  $\kappa = (P(\text{agreement}) - P(e)) / (1 - P(e))$ , where  $P(\text{agreement})$  is the fraction of objects which are in the same class for both observers (the main diagonal in the contingency table), and  $P(e)$  is the probability of random agreement, given the marginals (like in a Chi-square test). If the observers are in complete agreement the Kappa value  $\kappa = 1$ . If there is no agreement among the observers, other than what would be expected by chance ( $P(e)$ ), then  $\kappa = 0$ .

5.3 The standard Cohen's Kappa statistic only considers perfect agreement versus non-agreement. If one wants to take the degree of disagreement into account (for example with ordinal characteristics), one can apply a linear or quadratic weighted Kappa (Cohen, 1968). If we want to have a single statistic for all observers simultaneously, a generalized Kappa coefficient can be calculated. Most statistical packages, including SPSS, Genstat and R (package Concord), provide tools to calculate the Kappa statistic.

5.4 As noted, a low  $\kappa$ -value indicates poor agreement and values close to 1 indicate excellent agreement. Often scores between 0.6-0.8 are considered to indicate substantial agreement, and above 0.8 to indicate almost perfect agreement. If needed, z-scores for kappa (assuming an approximately normal distribution) are available. The criteria for experienced DUS experts could be more stringent than for inexperienced staff.

## 6. Trial design

6.1 If we have multiple observers in a trial, the best approach is to have one person observe one or more complete replications. In that case, the correction for block effects also accounts for the bias between observers. If more than one observer per replication is needed, extra attention should be given to calibration and agreement. In some cases, the use of incomplete block designs (like alpha designs) might be helpful, and an observer can be assigned to the sub blocks. In this way we can correct for the systematic difference between observers.

7. Example of Cohen's Kappa

7.1 In this example, there are three observers and 30 objects (plots or varieties). The characteristic is observed on a scale of 1 to 6. The raw data and their tabulated scores are given in the following tables.

| Variety | Observer 1 | Observer 2 | Observer 3 |
|---------|------------|------------|------------|
| V1      | 1          | 1          | 1          |
| V2      | 2          | 1          | 2          |
| V3      | 2          | 2          | 2          |
| V4      | 2          | 1          | 2          |
| V5      | 2          | 1          | 2          |
| V6      | 2          | 1          | 2          |
| V7      | 2          | 2          | 2          |
| V8      | 2          | 1          | 2          |
| V9      | 2          | 1          | 2          |
| V10     | 3          | 1          | 3          |
| V11     | 3          | 1          | 3          |
| V12     | 3          | 2          | 2          |
| V13     | 4          | 5          | 4          |
| V14     | 2          | 1          | 1          |
| V15     | 2          | 1          | 2          |
| V16     | 2          | 2          | 3          |
| V17     | 5          | 4          | 5          |
| V18     | 2          | 2          | 3          |
| V19     | 1          | 1          | 1          |
| V20     | 2          | 2          | 2          |
| V21     | 2          | 1          | 2          |
| V22     | 1          | 1          | 1          |
| V23     | 6          | 3          | 6          |
| V24     | 5          | 6          | 6          |
| V25     | 2          | 1          | 2          |
| V26     | 6          | 6          | 6          |
| V27     | 2          | 6          | 2          |
| V28     | 5          | 6          | 5          |
| V29     | 6          | 6          | 5          |
| V30     | 4          | 4          | 4          |

The contingency table for observer 1 and 2 is:

| O1\O2 | 1  | 2 | 3 | 4 | 5 | 6 | Total |
|-------|----|---|---|---|---|---|-------|
| 1     | 3  | 0 | 0 | 0 | 0 | 0 | 3     |
| 2     | 10 | 5 | 0 | 1 | 0 | 1 | 17    |
| 3     | 2  | 1 | 0 | 0 | 0 | 0 | 3     |
| 4     | 0  | 0 | 0 | 1 | 0 | 0 | 1     |
| 5     | 0  | 0 | 0 | 1 | 0 | 2 | 3     |
| 6     | 0  | 0 | 1 | 0 | 0 | 2 | 3     |
| Total | 15 | 6 | 1 | 3 | 0 | 5 | 30    |

The Kappa coefficient between observer 1 and 2,  $\kappa(O1,O2)$  is calculated as follows:

- $\kappa(O1,O2) = (P(\text{agreement between } O1 \text{ and } O2) - P(e)) / (1 - P(e))$  where:
- $P(\text{agreement}) = (3+5+0+1+0+2)/30 = 11/30 \approx 0.3667$  (diagonal elements)
- $P(e) = (3/30).(15/30) + (17/30).(6/30) + (3/30).(1/30) + (1/30).(3/30) + (3/30).(0/30) + (3/30).(5/30) \approx 0.1867$ . (pair-wise margins)
- So  $\kappa(O1,O2) \approx (0.3667-0.1867) / (1-0.1867) \approx 0.22$

This is a low value, indicating very poor agreement between these two observers. There is reason for concern and action should be taken to improve the agreement. Similarly the values for the other pairs can be calculated:  $\kappa(O1,O3) \approx 0.72$ ,  $\kappa(O2,O3) \approx 0.22$ . Observer 1 and 3 are in good agreement. Observer 2 is clearly different from 1 and 3 and probably needs additional training.

## 8. References

Cohen, J..(1960) A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20: 37-46.

Cohen, J. (1968) Weighted kappa: Nominal scale agreement provision for scaled disagreement or partial credit. Psychological Bulletin, 70(4): 213-220.

Bland, J. M. Altman D. G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement, Lancet: 307–310.

<http://www.seedtest.org/en/stats-tool-box-content---1--1143.html> (ISO 5725-2 based software)

[End of Annex and of document]