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**INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF
 PLANTS**
 Geneva

TECHNICAL COMMITTEE

**Fiftieth Session
 Geneva, April 7 to 9, 2014**

REVISION OF DOCUMENT TGP/8: PART II: SELECTED TECHNIQUES USED IN
 DUS EXAMINATION, SECTION 10: UNIFORMITY ASSESSMENT ON THE BASIS OF THE
 RELATIVE VARIANCE METHOD

Document prepared by the Office of the Union

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1. The purpose of this document is to present a proposal for revision of document TGP/8/1, Section 10: "Uniformity Assessment on the Basis of the Relative Variance Method".

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:

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| ANNEX II | Revised draft proposal for revision of document TGP/8 Section 10: "Uniformity Assessment on the Basis of the Relative Variance Method" |

BACKGROUND

4. The Technical Committee (TC), at its forty-eighth session, held in Geneva from March 26 to 28, 2012, considered a proposal for a revision of Section 10: Uniformity Assessment on the Basis of the Relative Variance Method on the basis of document TC/48/19 Rev. "Revision of document TGP/8: Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability", Annex XIV. The TC noted the comments of the Technical Working Party on Automation and Computer Programs (TWC) with regard to some of the assumptions of the method and noted that further investigations would be done by Australia with respect to those assumptions and the F value used in the calculations (see document TC/48/22 "Report on Conclusions" paragraph 65).

5. The TC agreed with the workplan for the development of TGP/8 presented in Annex XV to document TC/48/19 Rev., which indicated that Section 10: Uniformity Assessment on the Basis of the Relative Variance Method would be considered by the TWPs in 2012. 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" paragraphs 49 and 78).

COMMENTS BY THE TECHNICAL COMMITTEE IN 2013

6. The TC, at its forty-ninth session held in Geneva from March 18 to 20, 2013, considered document TC/49/27 "Revision of document TGP/8: Part II: Techniques Used in DUS Examination, Section 10: Minimum Number of Comparable Varieties for the Relative Variance Method". The TC noted the proposed amendments of revision of Section: 10 of document TGP/8, as set out in Annex II of document TC/49/27 (see document TC/49/41 "Report on the Conclusions" paragraphs 60 and 61).

7. The TC agreed to invite the expert from Australia to prepare a new draft of Section: 10 of document TGP/8 with a recommendation on the minimum number of comparable varieties, for consideration by the TWPs at their sessions in 2013. The Delegation of Australia explained that the minimum number was one (see document TC/49/41 "Report on the Conclusions" paragraph 62).

COMMENTS BY THE TECHNICAL WORKING PARTIES IN 2013

8. At their sessions in 2013, the TWO, TWF, TWV, TWC and TWA considered documents TWO/46/16, TWF/44/16, TWV/47/16, TWC/31/16 and TWA/42/16, respectively. The draft proposed revision of document TGP/8, section 10: "Minimum Number of Comparable Varieties for the Relative Variance Method" considered by the TWPs is reproduced in Annex I of this document. The following comments were made by the TWPs:

| | | |
|---------|---|-----|
| General | The TWO noted the comments made by the TWPs at their sessions in 2012 and the TC, at its forty-ninth session in 2013. The TWO agreed with the proposed amendments for revision of Section 10 of document TGP/8 and the new proposed guidance in paragraphs 10.2.2 and 10.6 to specify the minimum number of comparable varieties in the relative variance method (see document TWO/46/29 "Report", paragraph 36). | TWO |
| | The TWF noted the comments made by the TWPs at their sessions in 2012 and the TC, at its forty-ninth session in 2013. The TWF agreed with the proposed amendments for revision of Section 10 of document TGP/8 and the new proposed guidance in paragraphs 10.2.2 and 10.6 to specify the minimum number of comparable varieties in the relative variance method as set out in the Annex to document TWF/44/16 (see document TWF/44/31 "Report", paragraph 39). | TWF |

| | | |
|---|---|-----|
| | The TWV noted the comments made by the TWPs at their sessions in 2012 and the TC, at its forty-ninth session, held in 2013. The TWV agreed with the proposed amendments for revision of Section 10 of document TGP/8 and the new proposed guidance to specify the minimum number of comparable varieties in the relative variance method as set out in the Annex to document TWV/47/16 (see document TWV/47/34 "Report", paragraph 39). | TWV |
| | The TWA noted that the current thresholds in document TGP/8, Section 10 should be corrected, but agreed that the proposed text should not replace current paragraph 10.2.1. The TWA agreed that it would not be necessary to develop further guidance on the minimum number of comparable varieties in particular because it could cause confusion with the guidance provided in TGP/10, with regard to new types and species (see document TWA/42/31 "Report", paragraph 40). | TWA |
| Title of section 10.2 and other mentions to "threshold limit" | The TWC agreed that all mentions to "threshold limit" should be replaced by "threshold" including the title of section 10.2, which should read "10.2 Threshold for different sample sizes". The TWC proposed that the second sentence of paragraph 10.2.1 should read (see document TWC/31/32 "Report", paragraph 36): "For example, if the actual sample size of the number of comparable varieties is 60 1, and the number of comparable varieties is limited for that species sample size is 60 for that variety , then the threshold limit is 1.84 (df1 =60, df2 =60)". | TWC |

PROPOSAL

9. On the basis of the comments of the TWPs at their sessions in 2013, Annex II to this document contains the proposed revision of Section 10: "Uniformity Assessment on the Basis of the Relative Variance Method", prepared by the drafter (Mr. Nik Hulse, Australia).

10. *The TC is invited to consider the proposed revision of document TGP/8, Section 10: "Uniformity Assessment on the Basis of the Relative Variance Method", as set out in Annex II to this document.*

[Annexes follow]

DRAFT PROPOSAL FOR REVISION OF DOCUMENT TGP/8 SECTION 10: "MINIMUM NUMBER OF COMPARABLE VARIETIES FOR THE RELATIVE VARIANCE METHOD" CONSIDERED BY THE TC AND TWPS AT THEIR SESSIONS IN 2013

Note for Draft version

Underlining (highlighted) indicates proposed changes to document TGP/8/1

10. UNIFORMITY ASSESSMENT ON THE BASIS OF THE RELATIVE VARIANCE METHOD

10.1 Use of the relative variance method

10.1.1 The relative variance for a particular characteristic refers to the variance of the candidate divided by the average of the variance of the comparable varieties (i.e. Relative variance = variance of the candidate/average variance of the comparable varieties). The data should be normally distributed. The relative variance method may be applied to any measured characteristic that is a continuous variable irrespective of the method of propagation of the variety. Comparable varieties are varieties of the same type within the same or a closely related species that have been previously examined and considered to be sufficiently uniform (see document TGP/10, Section 5.2 "Determining acceptable level of variation").

10.1.2 In cross-pollinated varieties, a common recommendation in the UPOV Test Guidelines is to take 60 measurements per characteristic per variety. In essence, the variance ratio equates to the F statistic, and the tabulated value of F at $P = 0.01$ under $df_1 = 60$ (degrees of freedom of candidate) and $df_2 = \infty$ (degrees of freedom of comparable variety(ies)) is 1.47. $df_2 = \infty$ is chosen as a conservative estimate, as it is assumed that comparable varieties accurately represent the infinite number of possible comparable varieties for the species as a whole. Therefore, 1.47 is the threshold limit for cross-pollinated species with 60 measurements per characteristic per variety. For different sample sizes, a different F statistic should be used for the df_1 , although the df_2 should remain at ∞ .

10.2 Threshold limit for different sample sizes

10.2.1 However, when there is a limited number of comparable varieties available for a species, it is not practical to use a conservative estimate of $df_2 = \infty$. In those cases, it is recommended to use the actual sample size of the comparable varieties to estimate the value of df_2 . For example, if the actual sample size of the comparable varieties is 60, and the number of comparable varieties is limited for that species, then the threshold limit is 1.84. ($df_1 = 60$, $df_2 = 60$).

10.2.2 The minimum number of comparable varieties is in part determined by the total number of comparable varieties available within the relevant taxon. Where the available number of comparable varieties is very low, it is recommended that all are included. In cases where the number of comparable varieties is large, then the number includes those in the trial but may also take into consideration data from previous trials where the authority determines that the comparable varieties in the trial may not provide a representative estimate of the population variance for all comparable varieties. (see TGP/8.1 section 3.6.2.2 for guidance on expansion of variety by year tables). Further information on the use of previous empirical data to estimate variance is included in ASTM E122-09e1 "Standard Practice for Calculating Sample Size to Estimate, With Specified Precision, the Average Characteristic of a Lot or Process".

10.3 The relative variance test in practice

10.3.1 When the calculated relative variance is lower than the tabulated value of F then it is reasonable to assume that the variances are equal and the candidate variety is uniform in that particular characteristic. If the calculated relative variance is higher than the tabulated value of F, then the null hypothesis, that the varieties have equal variances, is rejected. The candidate variety would then be deemed to have a higher variance than the comparable varieties for that particular characteristic and, therefore, would not meet the uniformity criteria.

10.4 Example of relative variance method

Example

10.4.1 In a DUS trial, a cross-pollinated candidate variety is grown together with a number of varieties representing the required level of uniformity for all relevant characteristics. In order to illustrate the calculation of the relative variance, an example with 4 comparable varieties is given. The variance data on plant height measurements for the five varieties are presented in Table 1. For each variety, 60 plants were measured for plant height:

10.4.2 The number of observations per variety is the same ($n=60$); therefore, we can take the average variance of the comparable varieties as their pooled variance.

10.4.3 The average variance for comparable varieties is $(7.8 + 4.5 + 3.2 + 5.8)/4 = 5.32$

If the variance of the candidate variety is lower than the average variance of the comparable varieties then no further test is required. It can be deemed that the candidate variety is sufficiently uniform in the relevant characteristic. However, if the variance of the candidate variety is higher than the average variance of the comparable varieties then the variances need to be compared using the relative variance method.

Table 1: variances of candidate and comparable varieties for plant height data

| Candidate | Comparable variety 1 | Comparable variety 2 | Comparable variety 3 | Comparable variety 4 |
|-----------|----------------------|----------------------|----------------------|----------------------|
| 5.6 | 7.8 | 4.5 | 3.2 | 5.8 |

10.4.4 The relative variance for a particular characteristic refers to the variance of the candidate divided by the average of the variance of the comparable varieties.

Relative variance = variance of the candidate/average variance of the comparable varieties

$$= 5.6/5.32 = 1.05$$

10.4.5 For a sample size of 60, the threshold limit is 1.47; therefore, we can conclude that the candidate variety is sufficiently uniform for that characteristic.

10.4.6 This is a conservative estimate of the relative variance method using $df2 = \infty$. If the variety is found to be non-uniform using this conservative approach then the competent authority may consider whether additional approaches, such as using the actual sample of the comparable varieties for the estimation of $df2$, are appropriate to provide a more precise estimate of uniformity.

10.5 Relationship between relative variance and relative standard deviation

10.5.1 Sometimes in DUS trials, the uniformity data is presented in terms of standard deviations, not as variances. Mathematically there is a simple relationship between variance and standard deviation, as follows:

Standard deviation = square root of Variance

10.5.2 When making a decision on uniformity based on relative standard deviations, the same principle for acceptance or rejection applies for relative standard deviation; only the threshold limits are lower due to the square root of appropriate values. For example, for 60 samples the relative variance threshold is 1.47; however, for relative standard deviation the threshold is 1.21, which is the square root of 1.47.

10.6 References

ASTM Standard C33, 2003a, "Standard Practice for Calculating Sample Size to Estimate, With Specified Precision, the Average Characteristic of a Lot or Process", ASTM International, West Conshohocken, PA, 2011, DOI: 10.1520/EO122-09EO1, www.astm.org.

REVISED DRAFT PROPOSAL FOR REVISION OF DOCUMENT TGP/8 SECTION 10: UNIFORMITY ASSESSMENT ON THE BASIS OF THE RELATIVE VARIANCE METHOD

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10. UNIFORMITY ASSESSMENT ON THE BASIS OF THE RELATIVE VARIANCE METHOD

10.1 Use of the relative variance method

The relative variance for a particular characteristic refers to the variance of the candidate divided by the average of the variance of the comparable varieties (i.e. Relative variance = variance of the candidate/average variance of the comparable varieties). The data should be normally distributed. The relative variance method may be applied to any measured characteristic that is a continuous variable, irrespective of the method of propagation of the variety. Comparable varieties are varieties of the same type within the same or a closely related species that have been previously examined and considered to be sufficiently uniform (see document TGP/10, Section 5.2 "Determining acceptable level of variation").

In cross-pollinated varieties, a common recommendation in the UPOV Test Guidelines is to take 60 measurements per characteristic per variety. In essence, the variance ratio equates to the F statistic, and the tabulated value of F at $P = 0.01$ under $df_1 = 60$ (degrees of freedom of candidate) and $df_2 = \infty$ (degrees of freedom of comparable variety(ies)) is 1.47. $df_2 = \infty$ is chosen as a conservative estimate, as it is assumed that comparable varieties accurately represent the infinite number of possible comparable varieties for the species as a whole. Therefore, 1.47 is the threshold limit for cross-pollinated species with 60 measurements per characteristics per variety. For different sample sizes, a different F statistic should be used for the df_1 , although the df_2 should remain at ∞ .

10.2 Thresholds limit for different sample sizes

10.2.1 Different thresholds limits of F (at $P = 0.01$) should be applied for different sample sizes of the candidate variety. The df_1 will vary according to different sample sizes of the candidate variety. However, in all cases the df_2 will be considered to be ∞ , to cover the whole range of possible comparable varieties within a species - thus providing a conservative estimate of the threshold. Under these conditions and taking the relevant values from the F table, Table 1 shows the thresholds limits that would apply for different sample sizes of the candidate varieties. In the case of different sample sizes than those included in Table 1, the correct threshold limit should be used for the exact sample size.

Table 1: Thresholds limit for relative variance for some different sample sizes

| Sample size of candidate | Thresholds <u>limit</u> for relative variance |
|---------------------------------|--|
| 30 | <u>1.70</u> |
| 40 | <u>1.59</u> |
| 50 | <u>1.53</u> |
| 60 | <u>1.47</u> |
| 80 | <u>1.41</u> |
| 100 | <u>1.36</u> |
| 150 | <u>1.29</u> |
| 200 | <u>1.25</u> |

Source: Table of F published in 'Tables for Statisticians' Barnes & Noble, Inc. New York

10.2.2 For a given sample size, if the relative variance exceeds the threshold limit, the candidate variety will be deemed to be non-uniform for that characteristic.

10.3 The relative variance test in practice

10.3.1 When the calculated relative variance is lower than the tabulated value of F statistic presented in Table 1, for the relevant sample size, then it is reasonable to assume that the variances are equal and the candidate variety is uniform in that particular characteristic. If the calculated relative variance is higher than the tabulated value of F, then the null hypothesis, that the varieties have equal variances, is rejected. The candidate variety would then be deemed to have a higher variance than the comparable varieties for that particular characteristic and, therefore, would not meet the uniformity criteria.

10.4 Example of relative variance method

Example

10.4.1 In a DUS trial, a cross-pollinated candidate variety is grown together with a number of varieties representing the required level of uniformity for all relevant characteristics. In order to illustrate the calculation of the relative variance, an example with 4 comparable varieties is given. The variance data on plant height measurements for the five varieties are presented in Table 2. For each variety, 60 plants were measured for plant height:

Table 2: variances of candidate and comparable varieties for plant height data

| Candidate | Comparable variety 1 | Comparable variety 2 | Comparable variety 3 | Comparable variety 4 |
|-----------|----------------------|----------------------|----------------------|----------------------|
| 5.6 | 7.8 | 4.5 | 3.2 | 5.8 |

10.4.2 The number of observations per variety is the same (n=60); therefore, we can take the average variance of the comparable varieties as their pooled variance.

10.4.3 The average variance for comparable varieties is $(7.8 + 4.5 + 3.2 + 5.8)/4 = 5.32$

10.4.4 The relative variance for a particular characteristic refers to the variance of the candidate divided by the average of the variance of the comparable varieties.

$$\begin{aligned} \text{Relative variance} &= \text{variance of the candidate/average variance of the comparable varieties} \\ &= 5.6/5.32 = 1.05 \end{aligned}$$

10.4.5 Now, in Table 1, for a sample size of 60, the threshold limit is 1.47; therefore, we can conclude that the candidate variety is sufficiently uniform for that characteristic.

10.5 Relationship between relative variance and relative standard deviation

10.5.1 Sometimes in DUS trials, the uniformity data is presented in terms of standard deviations, not as variances. Mathematically there is a simple relationship between variance and standard deviation, as follows:

$$\text{Standard deviation} = \text{square root of Variance}$$

10.5.2 Therefore, when dealing with relative standard deviations, Table 1 needs to be modified to include the square roots of the threshold limits, which is presented in Table 3.

Table 3: Thresholds limit for relative standard deviations for some different sample sizes

| Sample size of candidate | Thresholds limit for relative standard deviations |
|--------------------------|---|
| 30 | <u>1.30</u> |
| 40 | <u>1.26</u> |
| 50 | <u>1.24</u> |
| 60 | <u>1.21</u> |
| 80 | <u>1.19</u> |
| 100 | <u>1.17</u> |
| 150 | <u>1.14</u> |
| 200 | <u>1.12</u> |

10.5.3 When making a decision on uniformity based on relative standard deviations, the examiner needs to use Table 3, instead of Table 1, to get the appropriate threshold ~~limits~~. The same principle for acceptance or rejection applies for relative standard deviation; only the thresholds ~~limits~~ are lower due to the square root of appropriate values. For example, for 60 samples the relative variance threshold is 1.47; however, for relative standard deviation the threshold is 1.21, which is the square root of 1.47.

[End of Annex II and of document]