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RELATIVE VARIANCE METHOD FOR THE ASSESSMENT OF UNIFORMITY

Document prepared by experts from Australia

Background

1. In a DUS examination, each relevant characteristic has to be assessed for uniformity. For many characteristics, uniformity is assessed by the "off-type method".

2. However, the off-type method is not appropriate in situations where identification of off-types is difficult or imprecise (e.g. for measured characteristics in cross-pollinated varieties) (see document TGP/10). In these circumstances, the "relative variance method[1]" could be used to assess uniformity. In Australia, the relative variance method is applied to any measured characteristic that is a continuous variable, irrespective of the method of propagation of the variety.

3. To date, little guidance has been provided on the application of the methodology. In particular, its use for self, or mainly self-pollinated varieties has not been elucidated.

4. The purpose of this paper is to outline how the relative variance method is used for varieties with different methods of propagation and sample numbers.

[1] Relative variance for a particular characteristic refers to the variance of the candidate divided by the average of the variance of the comparators.

Use of the relative variance method

Cross-pollinated varieties

5. In cross-pollinated varieties, a common recommendation[2] 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 comparator(s)) is 1.60. $df_2 = \infty$ is chosen as a conservative estimate, as it is assumed that comparator varieties accurately represent the infinite number of possible comparators for the species as a whole. Therefore, 1.6 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∞.

Vegetatively propagated and self-pollinated crops

6. The recommended sample size in Test Guidelines for vegetatively propagated and self-pollinated crops is usually smaller than 60. In vegetatively propagated varieties, sampling rates between 10 and 60 are common. For self-pollinated varieties, sampling rates between 30 and 60 are not uncommon.

7. Accordingly, to ensure that the appropriate threshold for uniformity is applied, the correct F- distribution must be used.

Threshold limit for different sample sizes

8. Different threshold 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 comparator varieties within a species - thus providing a conservative estimate of the threshold. Under these conditions and taking the relevant values from the F table, the following threshold limits (Table 1) would apply for different sample sizes of the candidate varieties:

[2] Where relevant Test Guidelines (TG) exist, the sampling regime included in the TG should be used.

Table 1: Threshold limit for relative variance for different sample sizes

Sample size of candidate	Threshold limit for relative variance
10	4.31
15	3.00
20	2.49
25	2.21
30	2.03
40	1.81
50	1.68
60	1.60
80	1.49
100	1.43
150	1.33
200	1.28

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

9. 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. When the candidate sample size falls between the sample sizes in Table 1, the next highest threshold limit is used, as it provides a conservative estimation. For example, for a sample size of 35, the threshold limit of 1.81 is used.

The relative variance test in practice

11. 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 comparator varieties for that particular characteristic and, therefore, would not meet the uniformity criteria.

Examples of relative variance method

Example 1

12. In a DUS trial, a cross-pollinated candidate variety was compared against 4 similar varieties of common knowledge with the variance data on plant height measurements presented in Table 2. For each variety, 60 samples were taken for plant height measurement:

Table 2: variances of candidate and comparators for plant height data

Candidate	Comparator 1	Comparator 2	Comparator 3	Comparator 4
5.6	7.8	4.5	3.2	5.8

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

14. The average variance for comparators is (7.8 + 4.5 + 3.2 + 5.8)/4 = 5.32

15. The relative variance for a particular characteristic refers to the variance of the candidate divided by the average of the variance of the comparators.

Relative variance = variance of the candidate/average variance of the comparators

= 5.6/5.32 = 1.05

16. Now, in Table 1, for a sample size of 60, the threshold limit is 1.60; therefore, we can conclude that the candidate variety is sufficiently uniform for that characteristic.

Example 2

17. In a DUS trial, a self-pollinated candidate variety was compared against 3 similar varieties of common knowledge with variance data on plant height measurements as presented in Table 3. For each variety, 30 samples were taken for plant height measurement:

Table 3: variances of candidate and comparators for plant height data

Candidate	Comparator 1	Comparator 2	Comparator 3
6.2	3.2	2.5	2.8

18. The number of observations per variety is same (n=30); therefore, we can take the average variance of the comparators as their pooled variance

19. The average variance for comparators is (3.2 + 2.5 + 2.8)/3 = 2.83

20. Relative variance = variance of the candidate/average variance of the comparators

= 6.2/2.83 = 2.19

21. Now, in Table 1, for a sample size of 30, the threshold limit is 2.03; therefore we can conclude that the candidate variety does not meet the uniformity criteria for that characteristic.

<u>Relationship between relative variance and relative standard deviation</u>

22. 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

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 4.

Table 4: Threshold limit for relative standard deviations for different sample sizes

Sample size of candidate	Threshold limit for relative standard deviations
10	2.08
15	1.73
20	1.58
25	1.49
30	1.42
40	1.35
50	1.30
60	1.26
80	1.22
100	1.20
150	1.15
200	1.13

23. When making a decision on uniformity based on relative standard deviations, the examiner needs to use Table 4, instead of Table 1, to get the appropriate threshold limits. 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 1.60; however, for relative standard deviation the threshold is 1.26, which is the square root of 1.60.

Conclusion

24. As the relative variance method depends largely on the variance of comparator varieties, care should be taken when selecting the comparators from the list of reference varieties. As with any statistical method, the examiner needs to consider the suitability of the reference varieties. For example, if one reference variety has an unusually large variance then the examiner should consider whether to include that data in the relative variance method or not.

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