E

TWC/22/11
ORIGINAL: English
DATE: June 4, 2004

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY ON AUTOMATION AND COMPUTER PROGRAMS

Twenty-Second Session
Tsukuba, Japan, June 14 to 17, 2004

INFORMATION TO BE CONSIDERED FOR THE DEVELOPMENT OF TGP/10 EXAMINING UNIFORMITY

Document prepared by the Office of the Union

1. The document UPOV/DATA/BEI/04/8 "Examining uniformity", which is included in the Annex to this document, was originally prepared by an expert from France as the content of a presentation at the Workshop on Data Handling, Beijing, June 9 to 11, 2004. Nevertheless, with the agreement of its author and the chairman of the TWC, the content of the document was considered useful for the development of document TGP/10 Examining Uniformity.

2. The TWC is invited to consider the content of document UPOV/DATA/BEI/04/8 "Examining Uniformity", and the possible inclusion of certain elements in the relevant sections of TGP/10 Examining Uniformity.

E

UPOV

UPOV/DATA/BEI/04/8
ORIGINAL: English
DATE: May 25, 2004

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKSHOP ON DATA HANDLING

ORGANIZED BY
THE INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS (UPOV)

IN COOPERATION WITH
THE STATE FORESTRY ADMINISTRATION OF CHINA,
THE MINISTRY OF AGRICULTURE OF CHINA AND
THE STATE INTELLECTUAL PROPERTY OFFICE OF CHINA

WITH THE FINANCIAL ASSISTANCE OF
THE MINISTRY OF AGRICULTURE, FORESTRY AND FISHERIES OF JAPAN

Beijing, June 9 to 11, 2004

EXAMINING UNIFORMITY

Document prepared by Mr. Sylvain Grégoire, GEVES, Guyancourt, France, and presented by Mr. John Law, Head DUS Statistics, NIAB, Cambridge, United Kingdom

EXAMINING UNIFORMITY

1. According to Article 6(1)(c) of the 1961, 1972 and 1978 Acts of the UPOV Convention, a variety is deemed uniform if it is "sufficiently homogeneous, having regard to the particular features of its sexual reproduction or vegetative propagation."

2. Article 8 of the 1991 Act of the UPOV Convention states that: "The variety shall be deemed to be uniform if, subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics."

3. The UPOV Convention links the uniformity requirement for a variety to the particular features of its propagation. This means that the absolute level of uniformity required for vegetatively propagated varieties, truly self-pollinated varieties, mainly self-pollinated varieties, inbred lines of hybrid varieties, cross-pollinated varieties, mainly cross-pollinated varieties, population, synthetic varieties, and hybrid varieties will, in general, be different.

- Where all the plants of a variety are very similar, and in particular for vegetatively propagated and self-pollinated varieties, it is possible to assess uniformity by the number of obviously dissimilar plants –"off-types"–that occur. (See document TC/34/5 Rev.).

- However, where the range of variation within a variety is larger, because of the features of its propagation and in particular for cross-pollinated, including synthetic, varieties the plants are not all very similar, and it is not possible to visualize which plants should be considered as atypical or "off-types." In this case, the uniformity can be assessed by considering the overall range of variation, observed across all the individual plants, to determine whether intra-varietal variation is similar to comparable varieties. (See document TWC/18/10).

4. For a given species, both approaches can apply. For instance in *raphanus sativus* L., for new hybrid varieties, all plants should be very much alike (Figure 1), while for older varieties developed from populations, differences between plants of the same variety are likely to occur (Figure 2).

Figure 1: Hybrid where plants are very similar.

Figure 2: Variety with differences between plants.

5. These two general approaches are explained below:

6. UPOV has proposed methods for handling data which are quantitative, where the data under both these approaches are quantitative.

7. The same principles can be applied when data are not quantitative.

8. The off-type approach can be used with any type of recorded characteristic, as long as the experts agree on the definition of an "off-type". Some statistical methods are available to assess uniformity relative to comparable varieties when data are not quantitative, but expert judgement is also used. The General Introduction defines off-type as follows:

> "6.4.1.1 Determination of Off-Types by Visual Assessment
>
> "A plant is to be considered an off-type if it can be clearly distinguished from the variety in the expression of any characteristic of the whole or part of the plant that is used in the testing of distinctness, taking into consideration the particular features of its propagation. This definition makes it clear that, in the assessment of uniformity, the standard for distinctness between off-types and a candidate variety is the same as for distinctness between a candidate variety and other varieties (see Chapter 5, section 5.5.2)."

9. The four graphs illustrate another way of showing the two types of approaches:

10. In Figures 3 and 4, the plants of the variety are very similar (100 plants, normally distributed: mean = 120 cm; standard deviation = 1 cm), but two plants ((a) and (b))are very different from the others on this characteristic.

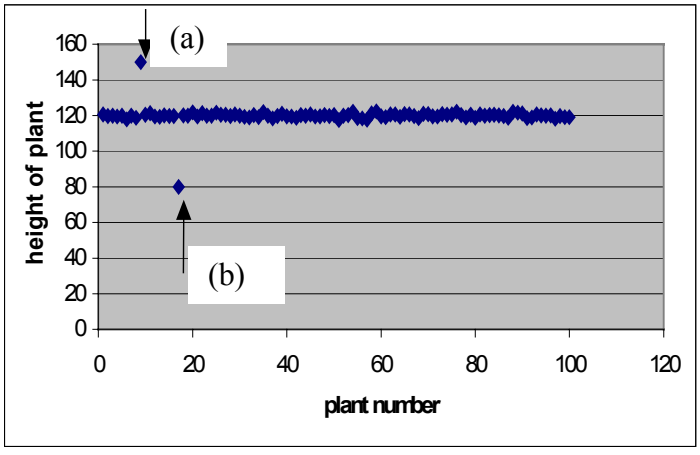
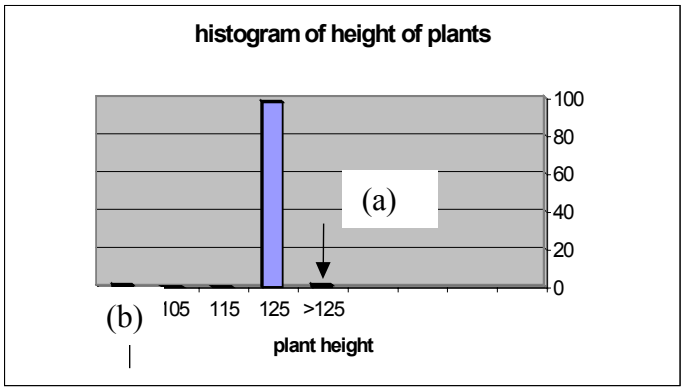


Figure 3: 100 plants measured, 1 point = 1 plant

Figure 4: corresponding histogram



Non deviating plants are alike.

11. In Figures 5 and 6, plant height varies from one plant to the other (100 plants, normally distributed: mean = 120 cm; standard deviation = 10 cm). Again, two plants ((c) and (d)) are very different from the others on this characteristic.

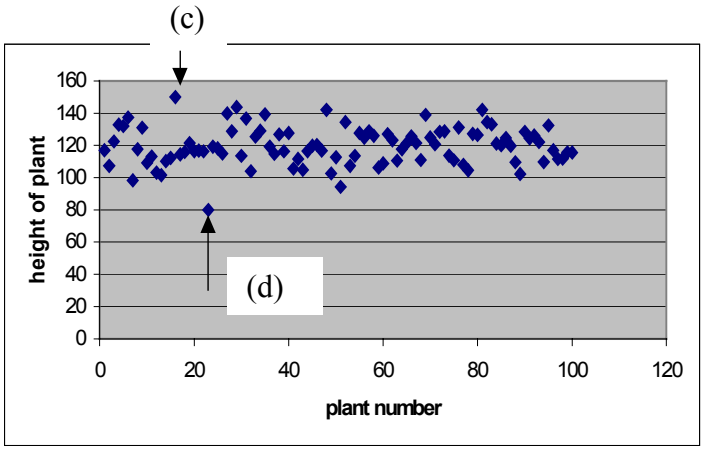
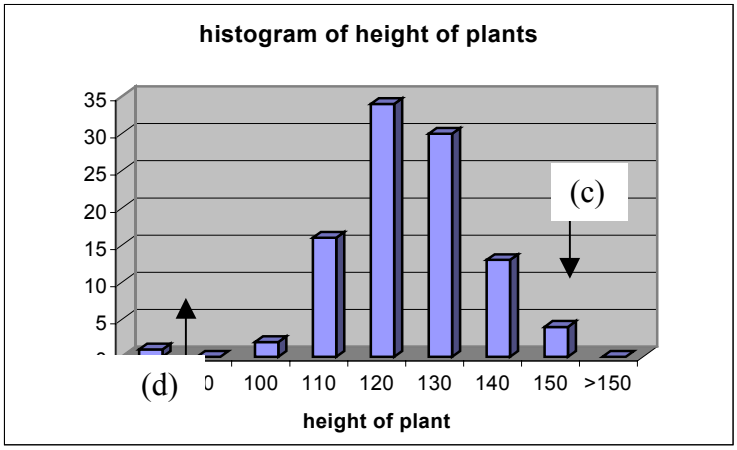


Figure 5: 100 plants measured, 1 point = 1 plant

Figure 6: corresponding histogram



The plants of the variety are not similar.

Approach 1: Relative Tolerance Limits

12. Cross-pollinated varieties, including mainly cross-pollinated varieties and synthetic varieties, generally exhibit wider variations within the variety than vegetatively propagated or self-pollinated varieties, and it is sometimes difficult to determine off-types.

13. Therefore, a fixed tolerance cannot be set, but relative tolerance limits, for the range of variation, are set by comparison with comparable varieties, or types, already known.

14. This means that the candidate variety should not be significantly less uniform than the comparable varieties.

15. The intra-varietal uniformity is assessed by computing the standard deviation (sd) from the values of the n plants in the plot.

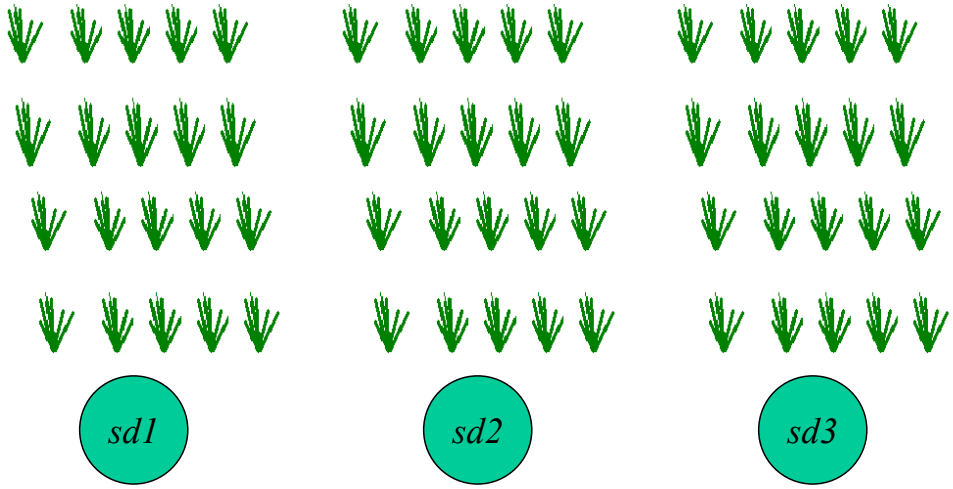
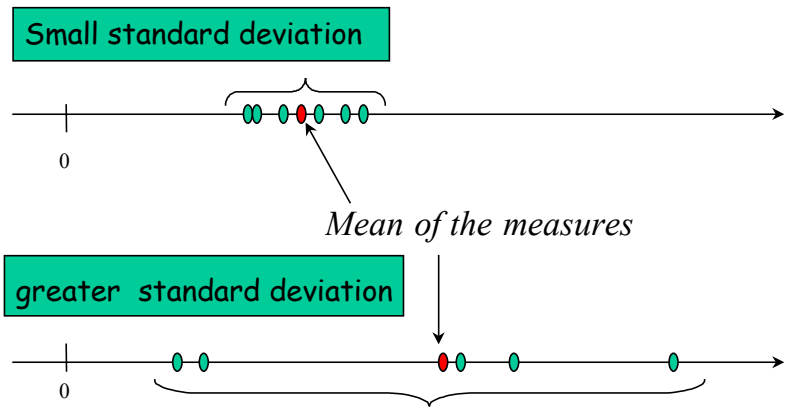


Figure 7: A measure of uniformity is obtained for each plot (sd1, sd2, sd3)

sd_j = *standard deviation in the plot j*
n_j = *number of plants in the plot j (20 in this example)*
y_{ij} = *value of the characteristic for plant number i (i from 1 to 20 in this example) in the plot j*
$\bar{y}_j$ = *mean of the n_j observations (20 in this example) of the characteristic in plot j*

$$sd_j = \sqrt{\sum_{i=1,20} (y_{ij} - \bar{y}_j)^2 / (n_j - 1)}$$



The axis represents the value of the characteristic or, in other words, the degree of expression. A green circle represents the value for one plant, the red circle represents the mean of the 20 values

Figure 8: The more alike the plants, the smaller the standard deviation

16. The average of the different values of uniformity (one sd value per plot) for each candidate variety is compared to the results obtained in the same way for the reference varieties in the same trials. In general, 2 or 3 independent growing cycles are used for the assessment. In many cases, there is one trial per year, but for some vegetables, for example, two independent growing cycles can be performed in the same year

17. The degree of expression may have an influence on the uniformity. For example, taller varieties are, in general, less uniform than shorter varieties. The COYU (Combined Over Years Uniformity) method compares the candidate to those reference varieties which have a similar level of expression. Thus a tall candidate is compared to the tall reference varieties, i.e. the reference varieties which are of a similar height but taller plus those which are of a similar height but shorter.

18. The degree of variability can be termed "homogeneity" or "heterogeneity" measurement, both address the same aspect and are used to assess uniformity. The computations give a tolerance for heterogeneity. Candidates showing levels of heterogeneity which are less or equal to the comparable reference varieties are accepted. Candidates with greater heterogeneity (less uniformity) than the tolerance limit (above the red line in Figure 10) will be refused for lack of uniformity. If the heterogeneity of a candidate is lower than the tolerance limit, but only slightly so (between the red and the gray line in Figure 10), another trial is appropriate before a final decision is taken.

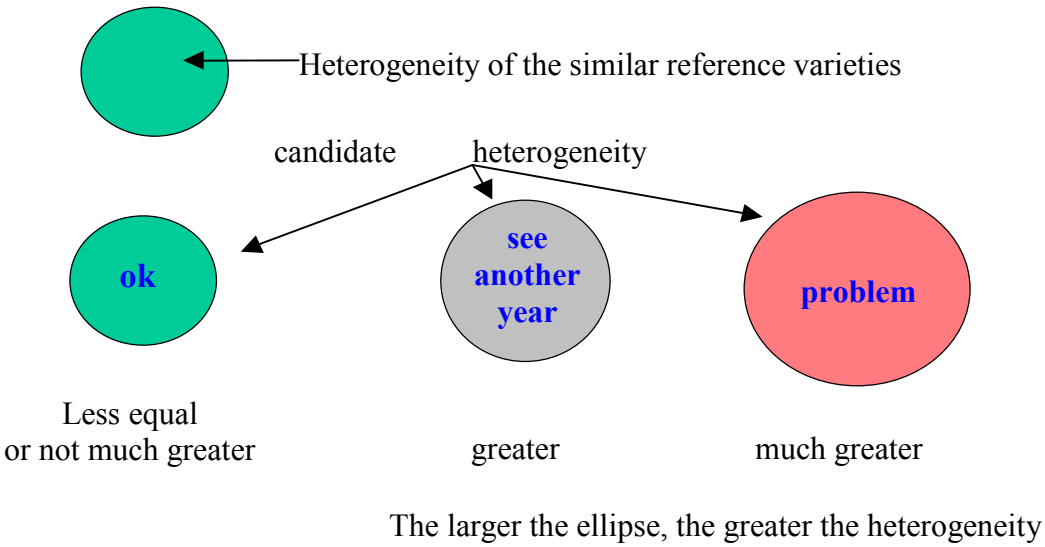


Figure 9: Candidates must not be more heterogeneous (less uniform) than comparable existing varieties

19. If we draw on a graph the values of heterogeneity for the candidate variety and the reference varieties (Y axis), in relation to the value of the characteristic (X axis), we see (Figure 10) that only the reference varieties which are similar to the candidate are used to determine what is "sufficiently uniform".

20. If the degree of expression has no influence on the variability, all the candidates will have the same tolerance limits for heterogeneity, i.e. the tolerance limit for heterogeneity would be a line parallel to the X axis.

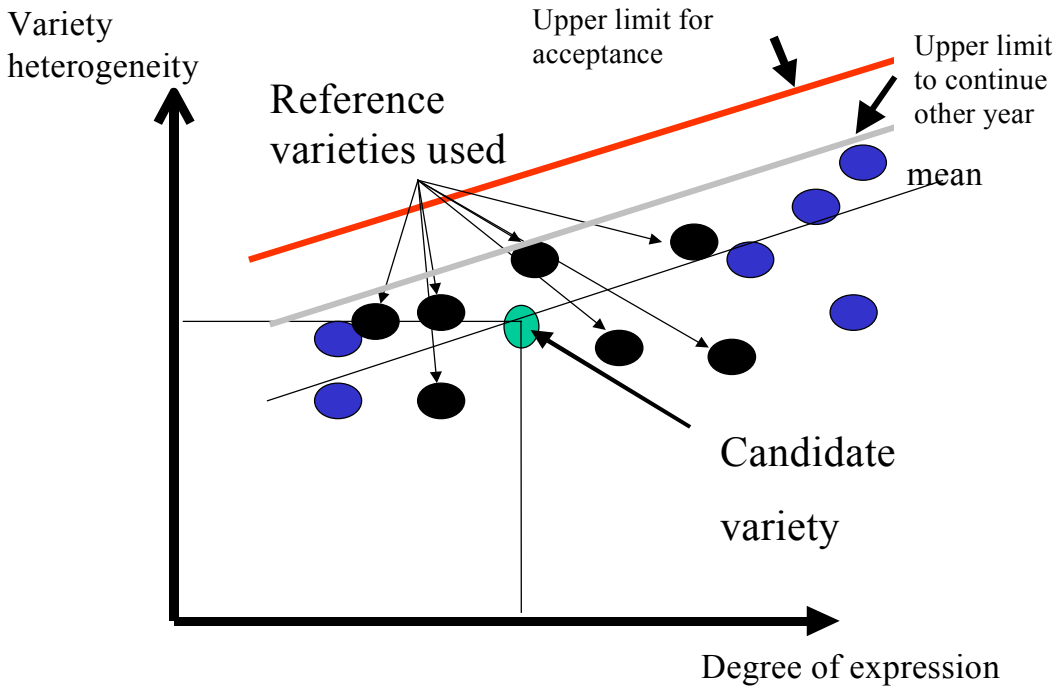


Figure 10: Tolerance takes into account relation [level of expression<-> variability] if it occurs

Approach 2: Off-types

21. For vegetatively propagated and self-pollinated varieties and for inbred lines of cross-pollinated varieties, the assessment of uniformity is, in general, based on the system of off-types. The experts must define what constitutes an off-type. It can be defined characteristic by characteristic, or globally.

22. For example, if branch(es) of a fruit-tree produce fruits which are not like the other fruits of the variety, experts might consider the tree to be an off-type.

23. In cereals, when plant height is considered, experts must know which limit they will admit as an acceptable difference, and when they will consider a given plant as "clearly distinguishable" from the other plants of the variety.

24. The Test Guidelines for the crop concerned contain a recommended maximum percentage of off-types for a given crop, in respect to its way of propagation or breeding. This percentage is called the "population standard" and is usually accompanied by an acceptance probability.

25. Based on this percentage, different scenarios can be used (see document TC/34/5 Rev.) with the appropriate number of plants to be checked and the decision rule.

26. For instance, if the population standard is defined as 1% and the probability level is 5%, then one can examine 60 plants and apply the following decision rule: if there are 0 to 2 off-types, the variety passes the test. If there are 3 off-types or more, the variety is not sufficiently uniform.

Figure 11: In the field, the experts usually mark the plants which they define as off-types by labels, flags, wool strings etc., to facilitate consultation and to monitor the plants throughout the duration of the trial

27. The acceptance probability is related to the probability of accepting a uniform variety meeting the population standard. If the population standard is 1% and the acceptance probability is 95%, it means that a variety with 1% of off-types has a 95% probability (95%=100%-5%) of being accepted.

28. One risk is that a uniform variety is wrongly rejected. This risk is commonly named the "breeder risk". The other risk is to accept a variety which is not uniform. This risk is commonly named the "user risk".

29. To describe these two risks the efficiency curve of a given method, with its decision rule, can be produced.

- The X-axis is the true percentage of off-types in the variety, which is an actual figure but cannot be known from sampling only a limited number of plants.

- The Y-axis is the probability of accepting the variety for the given number of plants (n) examined, and the decision rule applied (k = maximum tolerated off-types in n plants).

- AQL (Acceptable Quality Level) is the quality level which can be accepted often, i.e. the population standard in the Test Guidelines. To define "often", a level of probability is chosen, the "acceptance probability". The acceptance probability is 100% minus the alpha risk (α = risk of wrongly rejecting a uniform variety). Point A on the figure indicates the limit chosen to ensure that uniform varieties are accepted often.

- LQL (Low Quality level) is the level of quality which should be rejected often. To define "often" a level of probability is chosen, "power of the test". It is 100% minus the beta risk (β = risk of accepting a non uniform variety). Point B on the figure indicates the limit chosen to ensure that varieties lacking uniformity will be refused often.

30. The efficiency curve drawn on the graph is determined by the number of plants examined, and the decision rule, which is chosen according to the precision required.

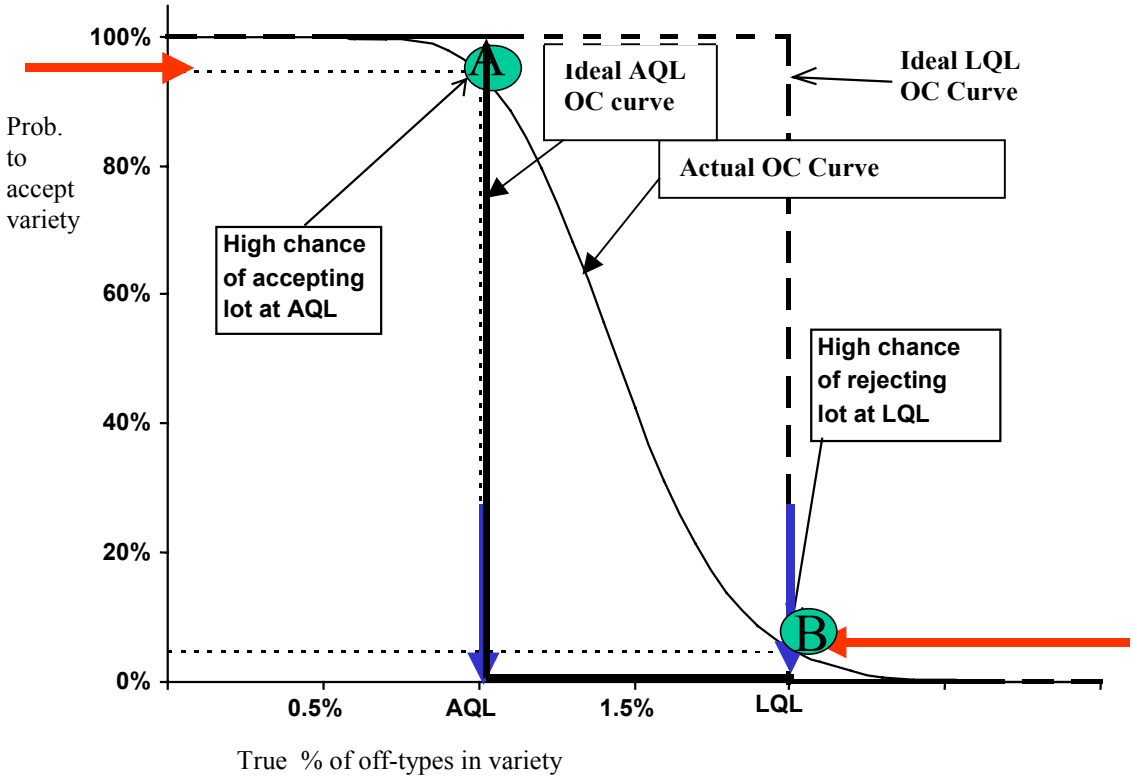


Figure 12: Efficiency curve for a given number of plants and decision rule

31. The greater the number of plants checked, the smaller will be the interval between AQL and LQL, and the steeper will be the decrease in the probability of accepting the variety along the X-axis (true percentage of off-types in the variety). The more plants checked, the more precise is the result, but practical considerations can limit the number of plants

available. For example, in some crops only 6 plants are available at the time of testing, while in other crops dozens, hundreds, sometimes thousands would be available.

DISCUSSION

There is a fundamental difference between the two approaches.

32. In the off-type approach, a separate check is made on each variety, looking only at the plants of the candidate variety without an individual comparison to other varieties. An acceptable number of off-types (population standard) is established for all varieties, on the basis of the features of their propagation.

33. In the relative tolerance limits approach, individual plants are used to establish the level of the differences between the plants of the candidate variety, and this level is compared to the level of difference between plants in comparable varieties.

Nevertheless the two approaches are not contradictory or exclusive, they are simply two different methods.

34. For some species only one approach is appropriate. In other species, the two approaches can be applied to different genetic structures within the species, for instance off-types for F1 hybrids, and relative tolerance limits for synthetic varieties. On a limited number of crops the choice is not obvious, and both can be used before the expert makes a decision.

In general, the same data are used to check for Distinctness, Uniformity, Stability, and to describe the variety.

35. A characteristic which is used to assess distinctness must also be examined for uniformity. This is often possible, and for instance COYD and COYU computations on quantitative measures use the same set of data for both Distinctness and Uniformity. In most cases, a “characteristic by characteristic ” approach is used. If the relative tolerance limits approach is used, uniformity is checked on each characteristic before a global decision is taken looking at the results for all characteristics. If the off-type approach is used, the number of off-types considering all the different characteristics is totalled. This means that a plant is considered as an off-type if it is obviously clearly distinguishable from the other plants of the variety for any characteristic.

Number of growing cycles

36. The result of the technical examination of a variety for the purposes of protection should be consistent. This is of particular relevance when considering that the period of protection of 20 or 25 years, at least, will be granted upon the basis of the technical examination. Therefore, one of the basic requirements, among others, that a characteristic should fulfil before it is used for DUS testing is that its expression be sufficiently consistent and repeatable in a particular environment. This can be achieved in both annual and perennial varieties by observations made on plantings in two different seasons or, in the case of other perennial varieties, by observations made in two different seasons after a single planting. However, in some circumstances the influence of the environment is not such that a second growing cycle

is required to provide assurance that the differences observed between varieties are sufficiently consistent. For example, if the growing conditions of the crop are controlled, such as in a greenhouse with regulated temperature and light, it may not be necessary to observe two growing cycles. In addition, the differences observed between varieties could be so clear that a second growing cycle may not be necessary. In both these circumstances, the features of propagation of the variety and the quality of the plant material will need to be taken into account. The individual Test Guidelines specify whether several independent growing cycles are required to show sufficient consistency, or whether, for certain species, the growing test could be made in one growing cycle.

37. The above-mentioned criteria are valid for the assessment of distinctness, uniformity and stability. Distinctness is not the subject of this paper and is included in other presentations at the Workshop. In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. Nevertheless, where appropriate, or in cases of doubt, stability can be tested, either by growing a further generation, or by testing a new seed or plant stock.

38. In Uniformity assessment, in the case of an off-type approach, sequential analysis could be used. It means that the results from the successive trials can be summed and compared to a tolerated limit for the number of off-types, as stated in advance for the different stages of test, in order to have consistent decisions along the number of trials available.

Number of plants examined

39. The number of plants examined has an influence on the precision of the work, on the stringency or leniency of the decision, and on the risk of taking the right or wrong decisions. This would be a whole subject in itself. To summarize, UPOV aims to enable studies in different countries to be conducted in a harmonized way, therefore, the sample size is often indicated in the Test Guidelines. If less plants are checked, there would be a loss of precision and an increased risk of risks of wrong decisions More precision is obtained with a larger number of plants but consistency of decision between offices might be lost.

[End of Annex and of document]