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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

Associated Document
to the
General Introduction to the Examination
of Distinctness, Uniformity and Stability and the
Development of Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

DOCUMENT TGP/9

"EXAMINING DISTINCTNESS"

Document prepared by the Office of the Union

to be considered by the

Technical Working Party for Vegetables (TWV),
at its thirty-eighth session, to be held in Seoul, from June 7 to 11, 2004

Technical Working Party on Automation and Computer Programs (TWC).
at its twenty-second session, to be held in Tsukuba, Japan, from June 14 to 17, 2004

Technical Working Party for Agricultural Crops (TWA),
at its thirty-third session, to be held in Poznan, Poland, from June 28 to July 2, 2004

Technical Working Party for Ornamental Plants and Forest Trees (TWO),
at its thirty-seventh session, to be held in Hanover, Germany, from July 12 to 16, 2004

Technical Working Party for Fruit Crops (TWF), at its thirty-fifth session,
to be held in Marquardt (Potsdam), Germany, from July 19 to 23, 2004

1. The document UPOV/DATA/BEI/04/3 “UPOV Test Guidelines and recording of characteristics”, which is included in the Annex to this document was originally prepared by an expert from Germany 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, the content of the document was considered useful for the e development of document TGP/9 Draft 1.

2. The Technical Working Parties are invited to consider the content of document UPOV/DATA/BEI/04/3 “UPOV Test Guidelines and recording of characteristics”, and the possible inclusion of certain elements in the relevant sections of TGP/9 Draft 1.

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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
UPOV TEST GUIDELINES AND RECORDING OF CHARACTERISTICS

Document prepared by an expert from Germany

UPOV TEST GUIDELINES AND RECORDING OF CHARACTERISTICS

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1. Introduction

1. The observation of the expression of characteristics is the basis for the assessment of distinctness, uniformity and stability (DUS) and for establishing a variety description. The dataset to be recorded depends on the demands of these aims. The assessment of DUS is based on characteristics which are defined in the UPOV Test Guidelines, in National Test Guidelines, or by the crop experts if such Test Guidelines have not been established.

2. The basic requirements that a characteristic should fulfill before it is used for DUS testing or producing a variety description are that its expression:

(a) results from a given genotype or combination of genotypes
(b) is sufficiently consistent and repeatable in a particular environment
(c) exhibits sufficient variation between varieties to be able to establish distinctness
(d) is capable of precise definition and recognition
(e) allows uniformity requirements to be fulfilled
(f) allows stability requirements to be fulfilled, meaning that it produces consistent and repeatable results after repeated propagation, or where appropriate, at the end of each cycle of propagation

(See document TG/1/3, Section 4.2 Selection of characteristics).

3. The choice of different statistical methods for the assessment of DUS depends on the data which are recorded for a characteristic. The data are influenced by the type of expression of a characteristic (qualitative, quantitative, pseudo-qualitative) as well as the method of observation (measurement, visual observation, number of records).

4. The type of expression of characteristics is defined in the "General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions for new Varieties of Plants" (document TG/1/3, Section 4.4), as follows:

"4.4 Types of Expression of Characteristics

To enable the appropriate use of characteristics in DUS testing, it is important to understand the different ways in which characteristics can be expressed. The following section identifies the different types of expression and considers their application in DUS testing.

4.4.1 Qualitative Characteristics

"Qualitative characteristics" are those that are expressed in discontinuous states (e.g. sex of plant: dioecious female (1), dioecious male (2), monoecious unisexual (3), monoecious hermaphrodite (4)). These states are self-explanatory and independently meaningful. All states are necessary to describe the full range of the characteristic, and every form of expression can be described by a single state. The order of states is not important. As a rule, the characteristics are not influenced by environment.

4.4.2 Quantitative Characteristics

"Quantitative characteristics" are those where the expression covers the full range of variation from one extreme to the other. The expression can be recorded on a one-dimensional, continuous or discrete, linear scale. The range of expression is divided into a number of states for the purpose of description (e.g. length of stem: very short (1), short (3), medium (5), long (7), very long (9)). The division seeks to provide, as far as is practical, an even distribution across the scale. The Test Guidelines do not specify the difference needed for distinctness. The states of expression should, however, be meaningful for DUS assessment.

4.4.3 Pseudo-Qualitative Characteristics

In the case of "pseudo-qualitative characteristics," the range of expression is at least partly continuous, but varies in more than one dimension (e.g. shape: ovate (1), elliptic (2), circular (3), obovate (4)) and cannot be adequately described by just defining two ends of a linear range. In a similar way to qualitative (discontinuous) characteristics – hence the term "pseudo-qualitative" – each individual state of expression needs to be identified to adequately describe the range of the characteristic."

5. The symbols QN, QL and PQ are used to indicate quantitative, qualitative and pseudo-qualitative characteristics, respectively.

6. There is a close relationship between the type of expression of characteristics, recording of data for the evaluation of characteristics and data for variety descriptions. The following presentation will demonstrate the importance of this relationship for the establishment and the application of Test Guidelines. The different methods of observation are demonstrated with various examples.

2. UPOV Test Guidelines

7. For many species UPOV has established specific Test Guidelines (TG) which represent an agreed and harmonized approach for the examination of new varieties. In conjunction with the basic principles contained in the General Introduction these TG should form the basis of the DUS tests.

8. Detailed guidance for the development of Test Guidelines is provided in TGP/7. If no UPOV Test Guidelines exist for a specific crop the same principles should be applied by national crop experts to develop appropriate national guidelines as elaborated in TG/1/3 and TGP/7.

2.1 Establishment of UPOV Test Guidelines

9. Individual UPOV Test Guidelines are prepared by the appropriate Technical Working Party, which is composed of goverment-appointed experts from each member of the Union with invited experts from other interested States and observer organizations. The leading expert drafts the Test Guidelines in close cooperation with all those experts of the TWPs who have expressed an interest, to ensure that the full extent of knowledge and expertise is refelected in the draft.

2.2 Recommendations in Test Guidelines

10. In order to obtain harmonized results in the DUS tests undertaken by all members of the Union and to establish harmonized variety descriptions, the Test Guidelines provide recommendations for the following elements:

- Minimum number of plants to be included in the test
- Number of replications
- Sample size for assessing distinctness
- Sample size for assessing uniformity and stability
- Number of independent growing cycles
- Table of characteristics which provides:
  - Clear definition of the characteristics including notes for description
  - Indication of type of expression of characteristic (in some TGs)
  - Indication of the method of observation (in some TGs)
  - Indication of developmental stage for defining characteristics and timing of observation (in some TGs)
  - Example varieties for defining characteristics and the harmonization of descriptions i.e. to provide calibration of the notes for local conditions
- Specific explanations for definition of characteristics, states of expression and method of observation (chapter 8 of the TGs).

11. The indication of the method of observation in the table of characteristics and the corresponding information about sample size as given in chapter 3.5 of the TG provide guidance on the number of records to be taken for each variety. It refers to the recording of data for the assessment of distinctness and/or uniformity. In general, the distinctness data are also used for variety descriptions. In some Test Guidelines, for some characteristics, it refers to the sample size for uniformity assessment. The different use of symbols in specific Test Guidelines should be considered when Test Guidelines are applied.

12. The recommended method of observation should be considered when establishing new UPOV Test Guidelines or national Test Guidelines. The recording of precise, reliable and consistent data is a key requirement for all further evaluations when planning a test. In the process of developing Test Guidelines it is important to take into account the relationship between the intended application of specific methods for DUS examination and the structure and quality of data which are necessary for these methods.

13. The method of observation in conjunction with the type of expression of characteristic enables the user of the TG to identify the appropriate recommendations for the examination of distinctness and uniformity in documents TG/1/3 “General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants”, TGP/9 “Examining Distinctness” and TGP/10 “Examining Uniformity”.

3. Methods for Observations

14. The expression of characteristics can be observed visually (V) or by measurement (M). Both types of observation can be used on either single plants (S) or plots/groups of plants (G).

The four resulting possibilities are listed below. The most appropriate method depends on different elements:

15. The following symbols are used in Test Guidelines to indicate the recommended method of observation:

MG: measurement of a group of plants or parts of plants
MS: measurement of a number of individual plants or parts of plants
VG: visual assessment of a group of plants or parts of plants
VS: visual assessment of a number of individual plants or parts of plants.

(i) Visual Assessment vs. Measurement

16. The choice of visual assessment or measurement will be influenced by the following factors:

- Type of expression of characteristic:
  Measurements - length, width, weight, time ... (quantitative characteristics)
  Visual assessment - growth habit, color, shape, glaucosity, hairiness ... (qualitative, quantitative or pseudo-qualitative characteristics)

- Genotypic and/or environmental variability between and within varieties determine if it is possible to record quantitative characteristics by visual assessment. Measurements provide a higher level of information and more precise data (objective units). Visual assessment requires more variation between, and less variation within, varieties (observations less precise, subjective units)

- Number of varieties in the collection: more precision may be necessary in order to distinguish a larger number of varieties. Measurements provide more precise data

- Resources (equipment, staff): visual assessment is usually less time consuming than measurements. Measurements for some characteristics may be partly automated (e.g. imaging). Different characteristics may be assessed simultaneously (e.g. thousand seed weight + kernel length; length + width of petals)

- Relation between workload and precision required.

17. Qualitative characteristics are, in general, assessed visually, whilst quantitative characteristics can be measured. However, visual assessment may also be appropriate for quantitative characteristics under certain conditions. If visual observation fulfills the requirements for the DUS assessment it is preferable because visual observations are quicker and less cost is involved compared to measurement.

18. Where there is doubt regarding the use of a visually assessed quantitative characteristic as the distinguishing characteristic in relation to another variety, it should be measured, if this is possible with reasonable effort.

(ii) Single Observation of a Group of Plants or. Observation on Individual Plants

19. An essential consideration for the number of observations per variety and growing cycle is the variation within and between varieties. The possibility of detecting off-types is another key consideration for the choice of method.

20. According to the General Introduction (document TG/1/3 Annex I, Chapter 6, section 6.4.1.1) off-types are defined as follows:

> "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."

21. For determination of the appropriate number of observations, it is necessary to consider if the recorded data will be used for assessment of distinctness, or for the assessment of uniformity or for the assessment of both. For distinctness, the "typical" expression of the varieties must be recorded, which may, after observation of the plot, be possible with only one record. The assessment of uniformity implies that it is necessary to observe single plants.

22. If there is only minor variation within varieties (excluding off-types), the expression of characteristics can be recorded by a single observation of a group of plants in order to provide sufficient data for assessment of distinctness as well as for the variety description. These conditions are fulfilled in most characteristics in self-pollinated and vegetatively propagated varieties and for most qualitative characteristics in cross-pollinated varieties.

23. If distinctness is based on a single observation of a group of plants, uniformity is assessed by visually observing the proportion of off-types. Every individual plant in the relevant sample is observed and classified as off-type or true-to-type.

24. If considerable genotypic and/or environmental variation occurs within plots it is necessary to observe individual plants in order to determine the mean expression as well as the variation within a variety. Distinctness is then assessed by comparing variety means calculated on the basis of the individual plant data, taking into account the random variation inherent in the variety means. If it is not possible to detect off-types, the same data may also be used for the assessment of uniformity and standard deviations are applied as uniformity standard. This is the normal situation for quantitative characteristics in cross-pollinated varieties.

4. Characteristics recorded by measurements

4.1 Morphological characteristics

25. Table 1 provides an example for recording measurements in self-pollinated varieties (barley) with very little intravarietal variation (single record) and in cross pollinated varieties (rye) with substantial plant to plant variation (records of individual plants).

26. In the case of barley, distinctness for the characteristic "Plant: length" is usually based on a single value for each variety. The replicated measurements within a plot determine the

mean plot value and the replications are not considered for further evaluations. If appropriate, the replications can be used to calculate a least significant difference for distinctness. Uniformity in this example is assessed on the basis of off-types, which are observed visually.

27. The data obtained from individual plant measurements in rye are used for the assessment of distinctness and uniformity.

Table 1: Examples for characteristics recorded by measurements

	Single measured value per variety	Measurements of individual plants
Example	Barley, TG/19/10, Characteristic 12 Plant: length (stem, ear and awns)	Rye, TG/58/6, Characteristics 10 + 11 Leaf next to flag leaf: length of blade Leaf next to flag leaf: width of blade
Recording of data	Replicated measurements in the plots and calculation of the mean value in order to determine a representative value for the plot (1-5 measurements in the plot depending on the variability within the plot) Measurement of all replications of the test and calculation of the mean value in order to determine a representative value for the variety under the specific year x location conditions Counting of off-types	60 plants per variety are recorded according to the Test Guidelines. The leaf next to flag leaf is collected from 60 plants (20 neighboring plants from each of 3 replicates). The plants at the beginning and the end of a row should be excluded. Measurement of leaf length and width in mm (e.g. using a ruler on the desk).
Distinctness assessment	on the basis of one value per variety (single measurements are not used for further evaluations)	on the basis of 60 single plant records per variety; same data for D & U (mean, SD)
Uniformity assessment	on the basis of off-types	
Description	mean value of variety transformed into note	mean value of variety transformed into note

4.2 Phenological characteristics

28. Phenological characteristics which are appropriate for DUS testing are mainly linked to time of flowering or maturity. They can be measured on a time scale (e.g. date of beginning of flowering). In order to take exact measurements for all varieties it may be necessary to go through the trial several times because the varieties reach the same developmental stage at different times and the characteristic can be observed only during a very restricted period for each variety.

29. The most precise way to record phenological characteristics is measurement on a time scale. If there are only few varieties in the trial and there are clear differences between the varieties it might be sufficient to observe all varieties at the same date on a quantitative scale (e.g. (1) very early to (9) very late). In that case the characteristic would be handled like any other visually observed quantitative characteristic (see 5.2).

30. Examples of some phenological characteristics are given in Table 2. Depending on the species and the local conditions it may be necessary to observe the trial three or more times per week to ensure that all varieties are recorded at the appropriate developmental stage. Measurements can be taken from plots or individual plants.

31. Single plant data for flowering time can be recorded in the following ways:

- The number of plants with inflorescences in each plot is recorded on each visit.
- The plants which began to flower after the last observation are equally distributed across the intervening period.

or

- For the plants which began to flower after the last observation the real date of flowering is estimated for each of the plants.

32. In both cases, the mean date per replicate and variety is calculated from the single plant data.

33. All calculations with data measured on a time scale must be handled cautiously, because the measured data can deviate from a normal distribution. For example, the scale can be distorted in cold or hot periods during flowering time. Sometimes it might be necessary to transform the measured data before further calculations can be made.

Table 2: Examples for phenological characteristics

Characteristic	Species	Explanation
Recording of plots (D on the basis of one measured value per variety; U on the basis of off-types)		
Time of beginning of flowering	Linseed	first flower open on 10% of plants
Time of ear emergence	Barley	first spikelet visible on 50% of plants
Time of anthesis	Maize	on middle third of main axis, 50% of plants
Time of ripening	Sweet Pepper	color change of fruits on 50% of plants
Time of maturity	Peas	Key 320 (hard dry seed)

Table 2: Examples for phenological characteristics (cont'd)

Recording of individual plants (D and U on the basis of individual plant records)		
Time of ear emergence	Rye	The number of plants which have reached stage 52 of the EUCARPIA Code should be recorded at two-day intervals. From these data the average time should be calculated.
Time of inflorescence emergence in year of sowing	Ryegrass	The date should be observed on each single plant. A plant is considered to have headed when the tip of three inflorescences can be seen protruding from the flag leaf sheath. From these data the mean date per plot and per variety is obtained. (Observations at least two times per week, more frequently if necessary)

4.3 Bulk samples

34. Some UPOV Test Guidelines include characteristics which are observed in bulk samples. These are characteristics like seed weight or content of certain compounds, where a plant-by-plant observation is inappropriate or impractical.

35. Uniformity is normally assessed on the basis of plant by plant observations. Therefore, specific rules will be developed for the handling of bulk samples for DUS assessment (see TGP/8 and TGP/9).

36. The number of plants per bulk, and bulks per variety should be fixed, as for example in the Test Guidelines for peas or rye for seed weight (Table 3).

Table 3: Examples for the measurement of seed weight in bulk samples

Species	Characteristic	Explanations
Rye	Grain: weight per 1000 grains	one harvested bunch is taken from each of the two row plots
Peas	Seed: weight	2 samples of 100 harvested seeds

5. Characteristics recorded by visual observation

37. For measured characteristics, the assessment of distinctness is based on the measured records, which are transformed into notes (qualitative scale) for a variety description.

38. If a characteristic is assessed visually, the observations are recorded as notes. Assessment of distinctness is based on these notes. The notes specified in the Test Guidelines should be used or, if no Test Guidelines exist the notes should be established following the rules as laid down in TGP/7. In order to standardize the states of expression as far as possible over different tests (adjustment to year and location effects), UPOV recommends the use of example varieties and/or provides additional explanations in Chapter 8 of the Guidelines.

39. Assessment of uniformity of visually observed characteristics is based on the proportion of off-types or on the variances of notes for individual plants.

5.1 Qualitative characteristics

40. In qualitative characteristics the states of expressions are self-explanatory and independently meaningful. Notes are provided for each of the states. Such characteristics are, in general, recorded by a single observation of a group of plants for distinctness and the off-type procedure is applied for uniformity.

Examples:

Barley - Lowest leaves: hairiness of leaf sheaths
(absent, present)

⇨ Distinctness - one visually assessed value per variety
⇨ Uniformity - off-types, fixed population standard

Fodder Beet - Root: color above ground
(white, green, yellow, orange, red, red purple)

⇨ Distinctness - one visually assessed value per variety
⇨ Uniformity - off-types, relative population standard

5.2 Quantitative characteristics

41. The expression of quantitative characteristics is typically recorded on a one-dimensional continuous, or discrete, scale. TGP/7 in the Guidance Notes 20 (GN 20) provides the following explanation:

> "3.3.1.1 As a general rule, states are formed in such a way that for the weak and strong expressions a reasonable word pair is chosen, for example:
>
> weak/strong
> short/long
> small/large
>
> 3.3.1.2 These word pairs are given Notes 3 and 7 and the intermediate state Note 5. The remaining states of the scale using Notes 1 to 9 are formed according to the following examples:

Note	**State**
1	very weak (or: absent or very weak)
2	very weak to weak
3	weak
4	weak to medium
5	medium
6	medium to strong
7	strong
8	strong to very strong
9	very strong

Note	***State***
1	very small (or: absent or very small)
2	very small to small
3	small
4	small to medium
5	medium
6	medium to large
7	large
8	large to very large
9	very large

However, it is not necessary to present all the 9 states in the Table of Characteristics.

42. In order to obtain comparable results from different testing locations the crop expert must "calibrate" his own scale of notes according to the recommended notes and adapt it for the specific situation (e.g. differentiation in the collection, environmental influence). Ideally the example varieties in the Test Guidelines can be used for this calibration, thereby ensuring that there is a universal calibration of variety descriptions produced by different authorities. If the example varieties given in the Test Guidelines are not applicable in the territory of the authority, a relevant set of example varieties should be developed to ensure calibration of the scale over the years. However, efforts should be made to harmonize calibration with other authorities, as far as possible.

43. Quantitative characteristics can be recorded by observation of a group of plants (mainly in self-pollinated and vegetatively propagated species) or by observations of single plants (mainly in cross-pollinated species).

Examples:

Wheat - Ear: glaucosity
(absent to very strong)

⇨ Distinctness - one visually assessed value per variety
⇨ Uniformity - off-types, fixed population standard

Ryegrass - Plant: growth habit
(erect to prostrate)

⇨ Distinctness - variety means calculated from visually assessed values for individual plants
⇨ Uniformity - relative uniformity based on variances

5.3 Pseudo-qualitative characteristics

44. Data for pseudo-qualitative characteristics are handled like qualitative characteristics. In general, they are recorded by observation of a group of plants for distinctness and the off-type procedure is applied for uniformity.

Radish - Radish: shape
(transverse elliptic, circular, elliptic, obovate, broad rectangular, rectangular, narrow rectangular, narrow obtriangular, iciclical)

⇨ Distinctness - one visually assessed value per variety
⇨ Uniformity - off-types, relative population standard.

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