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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

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SECTION 1: INTRODUCTION

From TGP/4: Sections 1.1 to 1.3

- 1.1 Article 7 of the 1991 Act of the UPOV Convention establishes that "a variety shall be deemed to be distinct if it is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of filing the application."
- 1.2 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), hereinafter referred to as "the General Introduction", states, with respect to common knowledge (see section 5.2.2), that:

"Specific aspects which should be considered to establish common knowledge include, among others:

- (a) commercialization of propagating or harvested material of the variety, or publishing a detailed description;
- (b) the filing of an application for the grant of a breeder's right or for the entering of a variety in an official register of varieties, in any country, which is deemed to render that variety a matter of common knowledge from the date of the application, provided that the application leads to the grant of a breeder's right or to the entering of the variety in the official register of varieties, as the case may be;
- (c) existence of living plant material in publicly accessible plant collections.

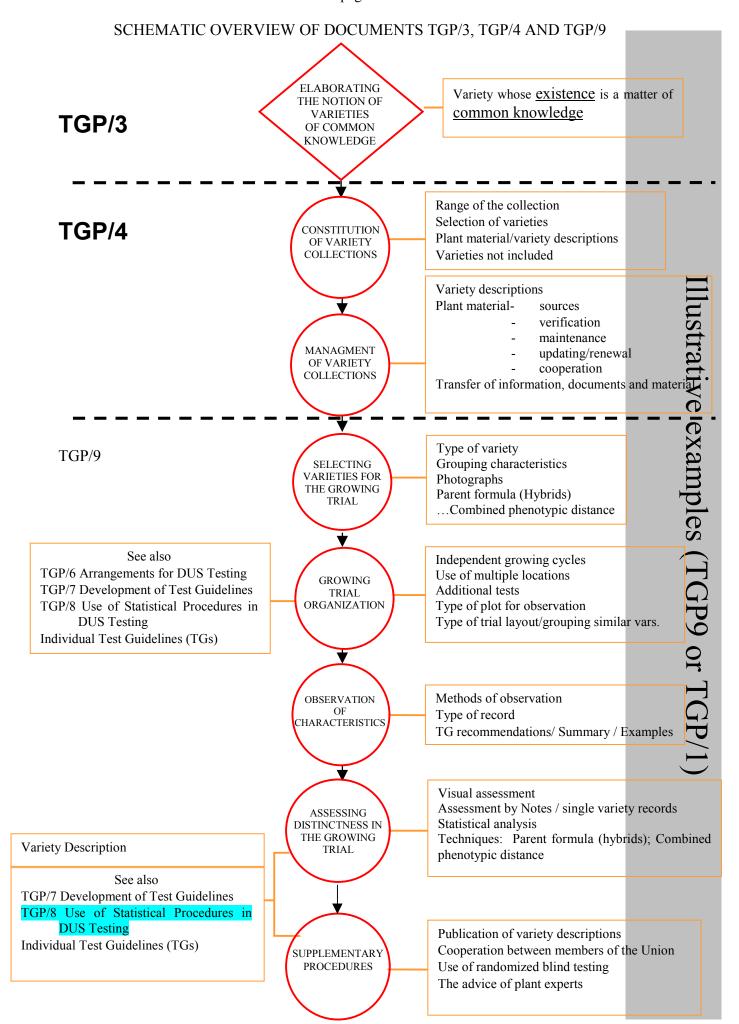
Common knowledge is not restricted to national or geographical borders."

Further information on varieties whose existence is a matter of common knowledge is provided in document TGP/3 "Varieties of Common Knowledge".

- 1.3 Although not exhaustive, and taking into account that these aspects have to be considered on a worldwide basis, it is clear that the list of varieties whose existence is a matter of common knowledge ("varieties of common knowledge") for a given species can be very large. Therefore, it is useful to employ a process to reduce the number of varieties of common knowledge which need to be included in a growing test or other trial for direct comparison against candidate varieties. That process involves, as a first step, defining a collection of varieties of common knowledge (a "variety collection") from within which:
 - (a) varieties which should be included in growing tests or other trials, as a part of the examination of distinctness, can be identified; and
 - (b) where required, the necessary living plant material of the varieties is available for inclusion in such tests and trials [, or supplementary procedures in place to avoid the need for a systematic individual comparison].

From TGP/4: Sections 2.2.1 to 2.2.3

- 1.4 The variety collection may not contain all varieties of common knowledge. For example, there may be situations where the authority cannot obtain living plant material of a variety, even if the existence of that variety is a matter of common knowledge. To address such situations the General Introduction (Chapter 5.3.1.2) states the following:
 - "... certain supplementary procedures may be developed to avoid the need for a systematic individual comparison. For example, the publication of variety descriptions, inviting comment from interested parties, or cooperation between members of the Union, in the form of an exchange of technical information, could be considered as supplementary procedures. However, such an approach would only be possible where the supplementary procedures, in conjunction with the other procedures, provide an effective examination of distinctness overall. Such procedures may also be appropriate for consideration of varieties of common knowledge, for which living plant material is known to exist (see section 5.2.2) but where, for practical reasons, material is not readily accessible for examination. Any such procedures are set out in document TGP/9, "Examining Distinctness."
- 1.5 In addition to the examples mentioned in the General Introduction, the making available of the list of varieties against which candidate varieties have been examined, and the making use of panels of experts, are other examples of supplementary procedures.
- 1.6 Article 21 of the 1991 Act of the UPOV Convention, requires the nullification of a right if it transpires that a variety did not meet the distinctness or novelty requirement. However, in order to maintain a high quality protection, such cases should remain the exceptions and establishment of variety collections and supplementary procedures, for addressing distinctness with regard to varieties of common knowledge not included in the variety collection, should be as robust as possible.
- 1.7 The definition of variety collections, is addressed in document TGP/4 "[Constitution and] Management of Variety Collections". The purpose of document TGP/9 is to provide guidance in the examination of distinctness in growing tests or other trials and on the use of supplementary procedures in the examination of distinctness. The following diagram presents a schematic overview of the process of examining distinctness as considered in documents TGP/3, TGP/4 and TGP/9:



SECTION 2: SELECTING VARIETIES FOR THE GROWING TRIAL

2.1 Introduction

- 2.1.1 A key step in the examination of distinctness is the selection of varieties of common knowledge, from within the variety collection (see document TGP/4), to be included in the growing test or other trials ("growing trials").
- 2.1.2 The General Introduction (see document TG/1/3, section 5.3.1.1 "Comparing varieties") explains that "It is necessary to examine distinctness in relation to all varieties of common knowledge. However, a systematic individual comparison may not be required with all varieties of common knowledge. For example, where a candidate variety is sufficiently different, in the expression of its characteristics, to ensure that it is distinct from a particular group (or groups) of varieties of common knowledge, it would not be necessary for a systematic individual comparison with the varieties in that group (or those groups).". This clarifies the possibility of reducing the number of varieties in the variety collection which need to be considered for inclusion in a growing trial and some factors which might be used in that process are discussed below.
- [2.1.3 The most important consideration in selecting varieties for inclusion in the growing trial is the identification of the most similar varieties of common knowledge. Once identified, at least the most similar varieties should be included in the variety collection and the growing trial. Other similar varieties may be excluded on the basis of grouping characteristics".]^a

2.2 Types of varieties

2.2.1 Document TGP/4 "Constitution and management of variety collections" explains that a variety collection may be limited to a type (or types) of varieties within a species or subspecies. Conversely, variety collections can comprise more than one type of variety and, in such cases, where it can be ensured that a candidate variety is distinct from all varieties of a particular type (or types) of varieties in the variety collection, it would not be necessary for a systematic individual comparison with the varieties of that type (or those types). Document TGP/4 "Constitution and management of variety collections" (see Section 2.1.1.2 *[cross ref.]*) identifies criteria with regard to types which might be used as follows:

From TGP/4: Sections 2.1.1.2

- (i) recognition of different types of variety within the relevant UPOV Test Guidelines, or by the establishment of separate Test Guidelines for different variety types within, for example, the same species;
- (ii) in the case of more than one geoclimatic zone for a given crop, the variety collection might be limited by taking into account certain physiological traits of the varieties (e.g. earliness, day length susceptibility, frost resistance, etc.) according to the climatic conditions for which it is adapted. Depending on the species, such criteria may or may not be useful;
- (iii) varieties within a particular group, identified by the use of grouping characteristics specified in the relevant Test Guidelines. With regard to groups of varieties, the General Introduction (Chapter 5.3.1.1) states the following:

- "It is necessary to examine distinctness in relation to all varieties of common knowledge. However, a systematic individual comparison may not be required with all varieties of common knowledge. For example, where a candidate variety is sufficiently different, in the expression of its characteristics, to ensure that it is distinct from a particular group (or groups) of varieties of common knowledge, it would not be necessary for a systematic individual comparison with the varieties in that group (or those groups)."
- (iv) types of varieties identified by the breeder, for example in the Technical Questionnaire

2.3 Grouping characteristics

2.3.1 Function

- 2.3.1.1 The selection of varieties to be grown in the trial with the candidate varieties is aided by the use of grouping characteristics.
- 2.3.1.2 The General Introduction sets out the functions of grouping characteristics (see document TG/1/3, section 4.8. Functional Categorization of Characteristics), as follows:
 - "1. Characteristics in which the documented states of expression, even where recorded at different locations, can be used to select, either individually or in combination with other such characteristics, varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness.
 - "2. Characteristics in which the documented states of expression, even where recorded at different locations, can be used, either individually or in combination with other such characteristics, to organize the growing trial so that similar varieties are grouped together."
- 2.3.1.3 Function 1 above identifies the role of grouping characteristics in selecting varieties for the growing trial.

2.3.2 Criteria

- 2.3.2.1 The General Introduction sets out the criteria (document TG/1/3, section 4.8 Functional Categorization of Characteristics) for the selection of grouping characteristics as follows:
 - "1. (a) Qualitative characteristics or
 - (b) Quantitative or pseudo-qualitative characteristics which provide useful discrimination between the varieties of common knowledge from documented states of expression recorded at different locations.

[...]"

2.3.2.2 The states of expression of the grouping characteristics for the candidate varieties need to be known before the growing trial in order to be able to use that information in

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selecting varieties for the growing trial. For that reason, information is requested in the Technical Questionnaire (TQ). Document TGP/7, "Development of Test Guidelines" (Guidance Notes 13.4) states that:

- "(a) Grouping characteristics selected from the Table of Characteristics should, in general, receive an asterisk in the Table of Characteristics and be included in the Technical Questionnaire.
- (b) TQ characteristics selected from the Table of Characteristics should, in general, receive an asterisk in the Table of Characteristics and be used as grouping characteristics. TQ characteristics are not restricted to those characteristics used as grouping characteristics;
- (c) Asterisked characteristics are not restricted to those characteristics selected as grouping or TQ characteristics."

Whilst TQ characteristics are, in general, included in the Technical Questionnaire in order to act as grouping characteristics, it should be noted that, in certain cases, characteristics may be included in the Technical Questionnaire for reasons other than providing information on grouping. Therefore, TQ characteristics should not be assumed to always be appropriate for grouping.

- 2.3.2.3 The identification of characteristics as useful grouping characteristics in the UPOV Test Guidelines is based on the information which is likely to be available from other members of the Union (asterisked characteristics) and to be requested from the breeder in the Technical Questionnaire (Technical Questionnaire characteristics). However, further characteristics may also be useful for grouping where the information available to the DUS examiner provides useful discrimination between varieties from documented states of expression for those characteristics, e.g. where the variety descriptions are obtained from a growing trial in the same location, such as can be the case from the first growing cycle where the DUS examination involves two growing cycles (see Section 2.3.6 *[cross ref.]*).
- 2.3.2.4 Where UPOV has developed Test Guidelines, these will provide useful grouping characteristics. However, grouping characteristics are provided in the Test Guidelines for two reasons, as specified in section 2.3.1.2 *[cross ref.]*. Therefore, the use of each grouping characteristic for excluding varieties from the growing trial, as opposed to its use for organizing the growing trial so that similar varieties are grouped together (see section 3.6.2 *[cross ref.]*), should be considered carefully.
- 2.3.2.5 In the absence of UPOV Test Guidelines, the criteria set out in this section should be used to identify suitable characteristics for selecting varieties for the growing trial.

2.3.3 Type of expression of grouping characteristics (QL, QN, PQ)

2.3.3.1 The use of grouping characteristics to identify those varieties in the variety collection which can be excluded from the growing trial is influenced by the type of expression of the characteristics chosen. In that respect it is recalled that grouping characteristics should be "qualitative characteristics or quantitative or pseudo-qualitative characteristics which provide useful discrimination between the varieties of common knowledge from documented states of expression recorded at different locations."

Qualitative characteristics

2.3.3.2 The use of qualitative characteristics for grouping is relatively straightforward because, as a rule, qualitative characteristics are not influenced by the environment (see document TG/1/3, section 4.4.1) and for qualitative characteristics, the difference between two varieties may be considered clear if one or more characteristics have expressions that fall into two different states in the Test Guidelines (see document TG/1/3, section 5.3.3.2.1). Therefore, in the case of qualitative characteristics, subject to the consistency of the observation on both the candidate variety and varieties in the variety collection, it is possible, in general, to exclude from the growing trial varieties which have a different state of expression to a candidate variety. For example, in the case of a qualitative characteristic: Leaf: variegation, with the states absent (Note 1): present (Note 9), it would be possible to exclude from the growing trial varieties in the variety collection which have no variegation (Note 1), for a candidate variety which is variegated (Note 9).

Quantitative and Pseudo-qualitative characteristics

- 2.3.3.3 In the case of quantitative and pseudo-qualitative characteristics, it is not possible to specify a general rule for discriminating between varieties on the basis of documented states of expression recorded at different locations. However, such characteristics can be used for grouping where there is a sufficient difference in the states of expression of varieties in the variety collection and the candidate variety, subject to the consistency of the observation on both the candidate variety and varieties in the variety collection. For example, in the case of a quantitative characteristic, e.g. "Plant: height", represented on a 1 to 9 scale it may, for example, be possible to exclude from the growing trial varieties in the variety collection which are very short (Notes 1 and 2) and very tall (Notes 8 and 9), if the candidate variety is of medium height (Note 5). In the case of a pseudo-qualitative characteristic, e.g. "Petal: color", with the states: white; yellow; green; pink; purple, it may, for example, be possible to exclude from the growing trial varieties in the variety collection which are yellow and green, if the candidate variety is pink. However, in the case of quantitative and pseudo-qualitative grouping characteristics, the range of varieties which can be excluded from the growing trial is determined by the influence of the environment on the states of expression, the difference in the environments where the varieties were observed and the consistency of the observation of the varieties in the variety collection.
- 2.3.3.4 The use of color characteristics for grouping is explored in document TGP/14 Section 2.3: Glossary of Technical, Botanical and Statistical Terms Used in UPOV Documents: Botanical Terms: Color: color characteristics *[cross ref.]*.

2.3.4 Combining grouping characteristics

As explained in Section 2.3.1.2 *[cross ref.]*, grouping characteristics "can be used to select, either individually or in combination with other such characteristics, varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness.". This clarifies that grouping characteristics can be used in combination to exclude varieties from the growing trial.

2.3.5 Utilization of grouping characteristics

2.3.5.1 The use of grouping characteristics can be a very effective means of reducing the number of varieties which need to be included in the growing trial. In particular, in cases

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where there are a small number of candidate varieties and a good number of grouping characteristics with high levels of discrimination, the number of varieties in the variety collection which can be excluded from the growing trial can be high. However, in other situations, in particular where there are large numbers of candidate varieties and few grouping characteristics with high levels of discrimination, the possibilities to exclude varieties from the growing trial may be limited because there may be candidate varieties of many or all of the groups defined by the grouping characteristics. In such cases, the grouping characteristics may still play an important role in organizing the growing trial so that similar varieties are grouped together (see Section 3.6.2 *[cross ref.]*).

2.3.5.2 Where it becomes apparent that information provided on the states of expression concerning the grouping characteristics for a candidate variety or a variety in the variety collection was not sufficiently accurate, taking into account the anticipated level of environmental and observation variation, it may be necessary to conduct a further growing trial containing the relevant varieties.

2.3.6 Grouping using information from a single location

- 2.3.6.1 Where information is obtained for all varieties from the same location, e.g. from the first growing cycle where the DUS examination involves two growing cycles, it may be possible to obtain a higher level of discrimination from the grouping characteristics and, in particular, for quantitative and pseudo-qualitative characteristics. For example, in the case of the quantitative characteristic in Section 2.3.3.3 *[cross ref.]*, "Plant: height", represented on a 1 to 9 scale, it was suggested that it might, for example, be possible to exclude from the growing trial varieties in the variety collection which are very short (Notes 1 and 2) and very tall (Notes 8 and 9), if the candidate variety was of medium height (Note 5) on the basis of information provided by the breeder in the Technical Questionnaire. However, on the basis of information obtained from the first growing cycle of a DUS examination, it might be possible to exclude, for example, varieties in the variety collection with Note 3 or Note 7 from the second growing cycle.
- 2.3.6.2 In the case of some perennial crops, e.g. fruit trees, a second growing cycle may be conducted using the trial established for the first growing cycle. In such cases, the notion of "excluding" varieties from the second growing cycle could mean that there would be no observation of the excluded varieties.
- 2.3.6.3 As noted in Section 2.3.2.3 *[cross ref.]*, the identification of characteristics as useful grouping characteristics in the UPOV Test Guidelines is based on the information which is likely to be available from other members of the Union (asterisked characteristics) and to be requested from the breeder in the Technical Questionnaire (Technical Questionnaire characteristics). However, further characteristics may also be useful for grouping where the information available to the DUS examiner provides useful discrimination between varieties from documented states of expression for those characteristics, e.g. where the variety descriptions are obtained from a growing trial in the same location, such as from the first growing cycle where the DUS examination involves two growing cycles. This is particularly relevant for quantitative and pseudo-qualitative characteristics, for which the states of expression are particularly influenced by the environment.

2.4 Photographs

- 2.4.1 Photographs can provide useful information for selecting varieties from within the variety collection to be included in the growing trial. In particular photographs may provide information on characteristics not included in the TQ. This may, for example, concern shapes and plant structures, which are not easy for applicants to describe by means of Notes in the Table of Characteristics and, therefore, might not be included as characteristics in section 5 of the TQ. In addition, the information provided in photographs on characteristics included in the TQ may be more discriminatory than that provided in section 5 of the TQ and may allow more varieties to be eliminated from the growing trial.
- 2.4.2 Document TGP/7 indicates that, where useful for the DUS examination, the Test Guidelines may require that a representative color photograph of the variety accompanies the information provided in the Technical Questionnaire. In these cases, it is recommended that guidance be provided by the authority to enhance the usefulness of the photograph (e.g. to include a metric scale in the picture, to define what parts of the plant should be included; light conditions, background color, etc).

2.5 Parent Formula of Hybrid Varieties

In the case of variety collections which contain hybrid varieties it may be appropriate to use the parent formula as a basis to select varieties for inclusion in the growing trial. The use of the parent formula requires that the difference between parent lines is sufficient to ensure that the hybrid obtained from those parents is distinct. Details of the parent formula technique are provided in TGP/8 *[cross ref.]*.

2.6 [Composite] / [Combined] / [Global] Phenotypic Distance

2.6.1 <u>Introduction</u>

[to be provided by experts from France based on document TWA/34/7 and the comments made by the Technical Working Party for Agricultural Crops (TWA) at its thirty-fourth session (see document TWA/34/14, paragraph 40)]

2.6.2 Methods

2.6.2.1 GAIA

- 2.6.2.1.1 The GAIA method, developed by experts from France, calculates a phenotypic distance between two varieties, which is a sum of distances for individual characteristics. For each genus or species, this system is calibrated to determine: (a) the weighting given to differences in each characteristic; and (b) the threshold, called "Distinctness plus" threshold, for the phenotypic distance used to eliminate varieties from the growing trial. The experience of crop experts is necessary to calibrate different parameters.
- 2.6.2.1.2 The GAIA software has been developed to automate these comparisons. Details of the GAIA method are provided in TGP/8 *[cross ref.]*.

2.6.2.2 Other Methods

[There are a range of other statistical methods in use in agricultural research that can be used in the examination of distinctness. Those include ANOVA and multiple range tests. Providing the underlying assumptions are met, those other statistical methods are as acceptable as the other methods mentioned in this section.]^b

2.7 Guidance and sources of information

- 2.7.1 The sections above identify factors which might be used for selecting varieties for inclusion in the growing trial. The following are useful sources of information in deciding on, for example, types of varieties and useful grouping characteristics:
 - (a) DUS experts from other members of the Union;
 - (b) breeders / applicants through:
 - (i) information provided in Section 5 of the Technical Questionnaire (Characteristics of the variety to be indicated by the applicant);
 - (ii) information provided in Section 6 of the Technical Questionnaire (Similar varieties and differences from these varieties);
 - (iii) information provided in Section 7 of the Technical Questionnaire (Additional information which may help in the examination of the variety); and
 - (iv) additional information
 - (c) plant experts
- 2.7.2 The process of selecting varieties from within the variety collection, including decisions on which of the factors above are appropriate, requires appropriate knowledge of the variety collection and the requirements for distinctness and it is recommended that, where necessary, guidance is sought from relevant experts, particularly experienced DUS examiners.
- 2.7.3 Some illustrations of the way in which the process can be applied are provided in:

[Note: The following is suggested for discussion purposes only]

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Option 1: Annex to TGP/9

Illustrative examples for particular crops of the process of: Defining the variety collection; Selecting varieties for the growing trial and organizing the growing trial; and Assessing distinctness in the growing trial.

The following table is intended to summarize the concept and content - the illustrations would be explained in full, i.e. not just in tabular form

	Ornamental (vegetatively propagated)	Maize	Ryegrass	Others
Defining the variety collection;	(according to species chosen)	to be explained	to be explained	
Selecting varieties for the growing trial and organizing the growing trial	types, grouping characteristics, photographs	parent formula, GAIA / alternative	cyclical growing of variety collection	
Assessing distinctness in the growing trial (plus any supplementary methods)		see Section	5.5	

Option 2: Section in TGP/1 General Introduction With Explanations

Illustrative examples for particular crops of the process of examining DUS:

Defining the variety collection;

Designing the DUS trial (including: one or two growing cycles / side-by-side comparisons or replicated trial);

Selecting varieties for the growing trial and organizing the growing trial; Assessing distinctness in the growing trial;

Assessing uniformity (off-types / relative tolerance limits)

SECTION 3: GROWING TRIAL ORGANIZATION

3.1 Number of independent growing cycles

3.1.1 A key consideration with regard to growing trials is to determine the appropriate number of growing cycles. In that respect, document TGP/7, Annex I: TG Template, section 4.1.2, states:

"4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles."

3.1.2 The Test Guidelines, where available, specify the recommended number of growing cycles. When making the recommendation, the experts drafting the Test Guidelines take into account factors such as the number of varieties to be compared in the growing trial, the influence of the environment on the expression of the characteristics, and the degree of variation within varieties taking into account the features of propagation of the variety e.g. whether it is a vegetatively propagated, self-pollinated, cross-pollinated or a hybrid variety.

3.2 The notion of independent growing cycles

- 3.2.1 As indicated in section 3.1 *[cross ref.]*, one means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles. The notion of independence is of particular relevance for the use of statistical procedures. In general, the assessment of independence is based on the experience of experts.
- 3.2.2 When varieties are grown in successive years and the layout of the plants in the trial is randomized (at least partly), the independence of the growing cycles is usually considered to be satisfied.
- 3.2.3 For some perennial crops, e.g. fruit trees, the same plants are examined over successive years. In such cases, the independence of growing cycles is considered to be satisfied.
- 3.2.4 In the case of plants grown in greenhouses, provided the time between two sowings is not "too short" and the layout of the plants in the trial is randomized (at least partly), two growing cycles can overlap and still be considered as independent.
- 3.2.5 Where two growing cycles are conducted in the same year and at the same time, a suitable distance or a suitable difference in growing conditions between two locations may satisfy the requirement for independence.

3.2.6 Where the two growing cycles are in the same location and the same year, a suitable time period between plantings may satisfy the requirement for independence.

3.3 Use of multiple locations in the examination of distinctness

Document TGP/7 "Development of Test Guidelines" (see Annex I, TG Template, section 3.2) clarifies that "Tests are normally conducted at one place". In cases where more than one place is used, the factors below should be taken into account:

3.3.1 Purpose

It may be considered appropriate to conduct tests at more than one place for the following purposes:

3.3.1.1 Minimizing the overall testing period

More than one location may be used as a means of achieving more than one independent growing cycle in, for example, the same year, as set out in section 3.2.5 *[cross ref.]*. This can, therefore, reduce the overall length of the testing period and facilitate a quicker decision.

3.3.1.2 Reserve trial

Authorities may designate a primary location, but organize an additional reserve trial in a separate location. In general, only the data from the primary location would be used, but in cases where that location failed, the reserve trial would be available to prevent the loss of one year's results.

3.3.1.3 Different agro-climatic conditions

Varieties of a different geographical origin may require different agro-climatic growing conditions. Varieties are distributed to the most appropriate location or, if the choice of the appropriate location is not obvious from the information known at the time of the receipt of the samples, to more than one location. Section 3.4 *[cross ref.]* Additional Tests addresses the situation where a variety needs to be grown in a particular environment for certain characteristics to be examined, e.g. winter hardiness.

3.3.2 Use of information from multiple locations

- 3.3.2.1 Where more than one location is used, it is necessary to establish decision rules to cover, for example, whether two varieties need to be distinct in only one location or in all locations.
- 3.3.2.2 [It is also necessary to define the way in which the information obtained in the centers would be used; e.g. whether it would be averaged over centers or whether each center would be considered individually.]

(To be redrafted with the assistance of TWA experts, including in particular experts from Australia, France and the United Kingdom. To address aspects such as: the need to develop rules for decisions on distinctness according to the features of the crop concerned; the risk of

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calculating averages for descriptions produced in different locations; and the importance of a final description produced at a single location (with exceptions for characteristics observed in Additional Tests).

3.4 Additional Tests

Document TGP/7 "Development of Test Guidelines" explains that, in addition to the main growing trial, additional tests, for examining relevant characteristics, may be established.

3.5 Type of plot for observation

The Test Guidelines may specify the type/s of plot for the growing trial (e.g. spaced plants, row plot, drilled plot, etc.) in order to examine distinctness as well as uniformity and stability.

3.6 Organizing the growing trial layout

3.6.1 Type of trial layout

The organization of the trial layout is, in the first instance, determined by whether the trial will have replicated plots and whether it will be randomized or whether it will be organized such that similar varieties are kept together in order to facilitate direct visual comparisons in the growing trial. The following sections focus on the situation where the growing trial is to be organized to facilitate direct visual comparisons. Information concerning replicated and randomized trial designs is provided in TGP/8 *[cross ref.]*.

3.6.2 Grouping of similar varieties

Section 2 *[cross ref.]* explains factors which might be used for selecting varieties for inclusion in the growing trial. Those factors can also be used to organize the growing trial such that similar varieties are grouped together in order to facilitate direct visual comparisons in the growing trial. The factors are:

3.6.2.1 Grouping characteristics

As noted in section 2.3.1.2 *[cross ref.]*, grouping characteristics are:

"[...]

"2. Characteristics in which the documented states of expression, even where recorded at different locations, can be used, either individually or in combination with other such characteristics, to organize the growing trial so that similar varieties are grouped together."

3.6.2.2 Photographs

See Section 2.4 [cross ref.]

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3.6.2.3 Parent Formula of Hybrid Varieties

See TGP/8 [cross ref.]

3.6.2.4 [Composite] / [Combined] / [Global] Phenotypic Distance

See TGP/8 [cross ref.]

SECTION 4: OBSERVATION OF CHARACTERISTICS

4.1 Method of observation (Visual or measurement)^c

4.1.1 Introduction

- 4.1.1.1 An important source of information on the observation of characteristics is the UPOV Test Guidelines, where those are available. In addition to the presentation of the characteristics and the states of expression in the Table of Characteristics (Chapter 7), further information is, where appropriate, provided in the Explanations on the Table of Characteristics (Chapter 8) in the form of explanations and/or illustrations. Document TGP/7 "Development of Test Guidelines" (see Guidance Note: GN 28) also explains that one of the purposes of including example varieties in the Test Guidelines is to illustrate the states of expression of a characteristic. However, the difficulty in selecting suitable example varieties which satisfy all the requirements for inclusion in the Test Guidelines means that a set of example varieties is not always provided for all characteristics.
- 4.1.1.2 In the absence of Test Guidelines, the principles set out in document TGP/7 "Development of Test Guidelines" provide suitable guidance with regard to the development and observation of characteristics for DUS testing.
- Suitable training is required to ensure that observations by a DUS examiner for a 4.1.1.3 characteristic are consistent and that repeatability between observers can be achieved. Such consistency and repeatability are important for the use of variety descriptions in the process of examining distinctness (see Section 2 *[cross ref.]*), notwithstanding the fact that variation in variety descriptions will also occur as a result of the influence of the environment. Document TGP/7 "Development of Test Guidelines" (see Guidance Note: GN 28) explains that, in addition to illustrating the states of expression of a characteristic, the other purpose of example varieties in the Test Guidelines is to provide the basis for ascribing the appropriate state of expression to each variety and, thereby, to develop internationally harmonized variety descriptions. It is difficult to identify example varieties which can satisfy that universal requirement and that is reflected in the fact that, as mentioned above, a set of example varieties is not always provided for all characteristics. However, identification of a suitable set of example varieties for all characteristics at a DUS trial center does not present the same difficulties and is an important measure for ensuring consistency and repeatability of observation at a national or regional level.
- 4.1.1.4 The expression of characteristics can be observed visually (V) or by measurement (M). Those two methods are explored in more detail below.

4.1.2 Visual observation

- 4.1.2.1 Visual observation (V) is an observation made on the basis of the expert's judgement. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. color charts).
- 4.1.2.2 Where they fulfill the requirements for the examination of DUS, visual observations can be used. They are generally quicker and cheaper than measurements but, because they are based on the expert's judgement, they have a particularly important

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requirement for training and experience to ensure that observations by a DUS examiner for a characteristic are consistent and that repeatability between observers can be achieved.

4.1.3 Measurement (M)

Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

4.1.4 <u>Selecting the Method of Observation</u>

The choice of the method for observations for the assessment of distinctness should take into account the following aspects:

(a) Type of expression of the characteristic:

Qualitative (QL) characteristics: qualitative characteristics are, in general, observed visually;

Quantitative (QN) characteristics: Quantitative characteristics can be measured or visually observed. The General Introduction explains that:

"5.4.1 In cases where there is very little variation within varieties, the determination of distinctness is usually on the basis of a visual assessment, rather than by statistical methods."

[...]

"5.5.2.2.1 Quantitative characteristics are not necessarily assessed by measuring or counting and can be assessed visually. Where there is doubt regarding the use of a normally visually assessed quantitative characteristic as the distinguishing characteristic in relation to another variety, it should be measured, if that is possible with reasonable effort."

Pseudo-qualitative characteristics: Pseudoqualitative characteristics are, in general, observed visually.

(b) Variability between and within varieties:

For the assessment of distinctness, visual observations are more suitable where there is sufficient variation between varieties, and a low level of variation within varieties. Measurements provide a higher level of information. The features of propagation determine the level of genotypic variation within varieties. Vegetatively propagated, truly self-pollinated and mainly self-pollinated varieties normally have relatively little variation within varieties. Within cross-pollinated and synthetic varieties, variation is normally greater than for self-pollinated and vegetatively propagated varieties, especially in quantitative and some pseudo-qualitative characteristics.

(c) Number of varieties in the collection [and in the growing trial]: more precision may be necessary in order to examine distinctness where there is a large number of varieties included in the growing trial. Measurements provide more precise data.

- (d) Resources (equipment, staff): visual observation is usually less time-consuming than measurements. However, measurements for some characteristics may be partly automated (e.g. imaging) and different characteristics may be measured simultaneously (e.g. thousand seed weight + kernel length; length + width of petals).
- (e) Relation between workload and precision required.

4.2 Type of record(s) for assessment of distinctness in the growing trial and for variety description purposes

4.2.1 Introduction

- 4.2.1.1 Records are taken from the growing trial for the purposes of assessing distinctness and/or for the generation of a variety description. The importance of inclusion of the variety, and its description, in the variety collection is explained in document TGP/4 "[Constitution and] Management of Variety Collections" [cross ref.] and Section 2 of this document [cross ref.].
- 4.2.1.2 The records taken for the assessment of distinctness may not be the same as those taken or retained for variety description purposes. For example, where visual assessment of distinctness is used, i.e. the assessment of distinctness is made by a direct visual comparison in the growing trial, the judgement of distinctness is not, as such, based on the records taken for the variety but the variety description is necessary for the variety collection. In general, where there is visual assessment of distinctness, a single variety record is kept for each characteristic for variety description purposes.
- 4.2.1.3 Observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded in the form of records for a number of single, individual plants or parts of plants (S). For observations of a group of plants (G), the single record may be obtained from either a single observation or by combining several observations.
- 4.2.1.4 The following sections consider the type of records which may be obtained and the way in which they may be used for the assessment of distinctness. For the purposes of examining uniformity, it is recalled that it is necessary to examine all the plants in the growing trial.

4.2.2 Single record of a group of plants or parts of plants (G)

- 4.2.2.1 If there is relatively little variation within varieties (excluding off-types) compared to the variation between varieties, 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 and pseudo-qualitative characteristics in cross-pollinated varieties.
- 4.2.2.2 As mentioned above, where visual assessment of distinctness is used, i.e. the assessment of distinctness is made by a direct visual comparison in the growing trial, the judgement of distinctness is not, as such, based on the records taken for the variety but the variety description is necessary for the variety description. In general, where there is visual

assessment of distinctness, a single variety record is kept for each characteristic for variety description purposes.

4.2.2.3 In the Test Guidelines, "Single record of a group of plants or parts of plants (G)" indicates that a single record (value or data) is retained. The single record may, for example, be in the form of: a Note (e.g. 1, 2, 3 etc.) corresponding to a state of expression in the Test Guidelines; more precise Notes (e.g. 1.1, 1.2, 1.3, etc.), a value (e.g. RHS Colour Chart reference number), a measurement (e.g. length (cm), weight (g), date (18-12-2005), count (58) etc.), an image etc. The single record may be obtained from an overall observation of the whole plot or on several visual observations (see example 1) or several measurements (see example 2).

Example 1

Several visual observations: Hairiness of lower side of leaf in barley (self-pollinated): leaves of several plants are observed;

Example 2

Several measurements: Plant: time of inflorescence emergence in meadow fescue, tall fescue (cross-pollinated); where at each observation date the average plot is expressed as: (1) boot swollen, (2) tip of inflorescence, (3) ½ of inflorescence emerged, (4) ½ of inflorescence emerged. The date of inflorescence emergence is the date at which the average plot has reached stage 2, which, if necessary, can be obtained by interpolation.

- 4.2.2.4 In most cases, "G" provides a single record per variety, in which case it is not possible or necessary to apply statistical methods for the assessment of distinctness. In some cases of G, e.g. where there are several repetitions or plots, or more than one growing trial, more than one record per variety may be obtained, in which case statistical methods can be applied.
- 4.2.2.5 Because, in most cases, "G" provides a single record per variety the same record used as the basis for distinctness could also used for the variety description. However, it may be appropriate to convert the record used for distinctness into another form for use as the variety description in the variety collection. For example, actual measurements taken from a growing trial might be the most accurate way of assessing distinctness, but for variety description purposes it may be more appropriate to convert those measurements into Notes calibrated against example varieties in order to make the information more meaningful when comparing descriptions obtained from different growing trials.

4.2.3 Records for a number of single, individual plants or parts of plants (S)

4.2.3.1 If there is considerable genotypic and/or environmental variation within varieties, 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. This is the normal situation for quantitative characteristics in cross-pollinated varieties.

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4.2.3.2 In the Test Guidelines, "Records for a number of single, individual plants or parts of plants (S)" indicates that records for individual plants, obtained by visual observation or measurements, are retained for the assessment of distinctness (see example 3).

Example 3

Plant height in grasses (cross-pollinated): each plant is measured and the value of each plant is then used for the assessment of distinctness.

- 4.2.3.3 Test Guidelines provide an indication of how many single, individual plants should be observed in the case of VS/MS (e.g. all observations should be made on $\{x\}$ plants or parts taken from each of $\{x\}$ plants).
- 4.2.3.4 In cases where records for individual plants, obtained by visual observation or measurements, are retained for the assessment of distinctness it may be necessary to convert that data into another form for variety description purposes as far as the variety collection is concerned. It may, for example, be appropriate to convert that individual plant data for the variety into a single record (e.g. Note), calibrated against example varieties, in order to make the information more meaningful when comparing descriptions obtained from different growing trials.

4.3 Recommendations in the Test Guidelines

The indication used in some the Test Guidelines for the recommended method of observation and type of record for the assessment of distinctness, is as follows:

"MG: single record for a group of plants or parts of plants based on measurement(s)

MS: records for a number of single, individual plants or parts of plants obtained by measurement

VG: single record for a group of plants or parts of plants based on visual observation(s)

VS: records for a number of single, individual plants or parts of plants obtained by visual observation.

For observations of a group of plants (MG, VG), the single record may be obtained from either a single observation or by combining several observations.".

4.4 Summary

The following table summarizes the common method of observation and type of record for the assessment of distinctness and/or variety description, although there may be exceptions:

	Type of expression of characteristic		
Method of propagation of the variety	QL	PQ	QN
Vegetatively propagated	VG	VG	VG /MG/MS
Self-pollinated	VG	VG	VG/MG/MS
Cross-pollinated	VG/(VS*)	VG/(VS*)	VS/VG/MS/MG
Hybrids	VG/(VS*)	VG/(VS*)	**

^{*} records of individual plants only necessary if segregation is to be recorded

4.5 Examples

See proposal in Section 2.7.3 (Option 2 could incorporate the following type of example retained from TGP/9 Draft 4, which addresses both distinctness and uniformity)

- 4.3.2.1 The table below provides an example for recording measurements in self-pollinated varieties (barley) with very little within-variety variation (single record: MG) and in cross pollinated varieties (rye) with substantial plant-to-plant variation (records of individual plants: MS).
- 4.3.2.2 In the case of barley, distinctness for the characteristic "Plant: length" is usually based on a single record 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 (LSD) for distinctness. Uniformity in this example is assessed on the basis of off-types, which are observed visually.
- 4.3.2.3 The data obtained from individual plant measurements in rye are used for the assessment of distinctness and uniformity.

^{**} to be considered according to the type of hybrid (see section 4.3.3).

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	Single record per variety	Records of individual plants
	(MG)	(MS)
Example	Barley, document TG/19/10, Characteristic 12: Plant: length (stem, ear and awns)	Rye, document 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 plot 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 plot replications of the test and calculation of the overall mean value in order to determine a representative value for the variety under the specific year x location conditions Counting of off-types	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
Distinctness assessment	on the basis of direct comparison or one record 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

SECTION 5: ASSESSING DISTINCTNESS BASED ON THE GROWING TRIAL

5.1 Introduction

The process of determining which varieties of common knowledge to include in the variety collection and which varieties in the variety collection to include in the growing trial are considered in document TGP/4 "[Constitution and] Management of Variety Collections" *[cross ref.]* and Section 2 of this document *[cross ref.]*, respectively. This section considers the assessment of distinctness based on the growing trial and identifies certain techniques which can be used in the assessment of distinctness.

5.2 Approaches for assessing distinctness

5.2.1 Introduction

- 5.2.1.1 Approaches for assessment of distinctness based on the growing trial can be summarized as follows:
 - (a) Visual assessment: side-by-side visual comparison in the growing trial;
 - (b) Assessment by Notes / single variety records: the assessment of distinctness is based on the recorded state of expression of the variety for a characteristic;
 - (c) Statistical analysis of growing trial data: the assessment of distinctness is based on a statistical analysis of the data obtained from the growing trial. This approach requires that, for a characteristic, there are a sufficient number of records for a variety.
- 5.2.1.2 The choice of approach for the assessment of distinctness will depend on the method of observation and type of record (VG, MG, VS or MS), which is influenced by the features of propagation of the variety and the type of expression of the characteristic. The common situations are summarized by the table in Section 4.4 *[cross ref.]*. The purpose of the following sections is to consider how the assessment of distinctness is conducted for those different situations.

5.2.2 Visual assessment

5.2.2.1 Visual assessment means that the assessment of distinctness is based on a direct visual comparison of varieties, side-by-side in the growing trial. This approach requires that the characteristics can be observed visually and indicates that the expression of the characteristic for a variety can be represented by a single record. It also requires that all similar varieties can be the subject of a direct side-by-side comparison in the growing trial. Such a requirement can be difficult to meet if the growing trial contains a large number of varieties and where there are limited possibilities for ensuring that all similar varieties are grouped together in the growing trial

- 5.2.2.2 Visual assessment is based on visual observation and, as explained in Section 4.1.2.2 *[cross ref.]*, because such observations are based on the expert's judgement, there is a particularly important requirement for training and experience.
- 5.2.2.3 As indicated in the table in Section 4.4 *[cross ref.]*, visual assessment is the most common approach for the assessment of distinctness for qualitative and pseudo-qualitative characteristics and is also commonly used for quantitative characteristics, particularly for vegetatively propagated varieties and self-pollinated varieties.

Qualitative (QL) characteristics

- 5.2.2.4 The General Introduction provides guidance on whether a difference between two varieties can be considered to be clear in the case of qualitative characteristics (see document TG/1/3).
 - 5.3.3.2.1 Qualitative characteristics: "In qualitative characteristics, the difference between two varieties may be considered clear if one or more characteristics have expressions that fall into two different states in the Test Guidelines. Varieties should not be considered distinct for a qualitative characteristic if they have the same state of expression."
- 5.2.2.5 Thus, varieties which have different states of expression for the same qualitative characteristic can be considered to be distinct. Conversely, varieties which have the same state of expression for a qualitative characteristic should not be considered to be distinct for that characteristic

Pseudo-qualitative (PQ) characteristics

- 5.2.2.6 The General Introduction provides guidance on whether a difference between two varieties can be considered to be clear in the case of pseudo-qualitative (PQ) characteristics:
 - 5.3.3.2.3 Pseudo-qualitative characteristics: "A different state in the Test Guidelines may not be sufficient to establish distinctness [...]. However, in certain circumstances, varieties described by the same state of expression may be clearly distinguishable."
- 5.2.2.7 The assessment of whether a pair of varieties are distinct needs to be judged according to the variation within varieties compared to the variation between varieties. In cases where there is very little variation within varieties, the determination of distinctness is usually on the basis of a visual assessment.

Quantitative characteristics

- 5.2.2.8 The General Introduction explains that:
 - "5.5.2.2.2 A direct comparison between two similar varieties is always recommended, since direct pairwise comparisons are the most reliable. In each comparison, a difference between two varieties is acceptable as soon as it can be assessed visually and could be measured, although such measurement might be impractical or require unreasonable effort."
- 5.2.2.9 The table in Section 4.4 *[cross ref.]* indicates that visual assessment may be suitable for all types of varieties. In the case of vegetatively propagated and self-pollinated varieties, there is relatively little variation within varieties and visual assessment of distinctness is

particularly suitable. However, where the range of variation within a variety is larger, because of the features of its propagation, and in particular for cross-pollinated and some types of hybrid varieties, determining distinctness on the basis of visual assessment requires particular care.

5.2.3 Assessment by Notes / Single variety records

- 5.2.3.1 Assessment by Notes / single variety records means that, for a particular characteristic, the assessment of distinctness is based on the recorded state of expression of a variety, obtained from the growing trial. In the case of Notes, the record is the Note corresponding to a particular state of expression in the relevant Test Guidelines. The different types of single records other than Notes are explained in Section 4.2.2.3 *[cross ref.]*. The Notes / single variety records approach can be used for characteristics which are visually observed or measured, but requires that the expression of the characteristic for a variety can be represented by a single record for the purpose of the assessment of distinctness. In most cases, when a single record is obtained by visual observation or measurement of a group of plants (VG / MG), this results in a single record per variety. However, in some cases, e.g. where there are several repetitions or plots, or more than one growing trial, more than one record per variety may be obtained, in which case statistical methods may be appropriate (see Section 5.2.4 *[cross ref.]*)
- 5.2.3.2 Where the requirements for distinctness assessment by Notes / single variety records are met it would usually also be possible to make a visual assessment. However, a visual assessment requires that varieties are the subject of a direct visual comparison, side-by-side, in the growing trial. In the case of assessment by Notes / single variety records, such proximity is not required, which is a particular advantage where the growing trial contains a large number of varieties and where there are limited possibilities for ensuring that all similar varieties are grouped together in the growing trial. On the other hand, because the varieties are not the subject of a direct visual side-by-side comparison, the difference required between varieties as a basis for distinctness is, with the exception of qualitative characteristics (see below), somewhat greater. The requirements for distinctness on the basis of Notes / single variety records are explained below:

Qualitative (QL) characteristics

- 5.2.3.3 The situation for qualitative characteristics concerning assessment by Notes / single records is the same as for visual assessment and, as explained in Section 5.2.2.4 *[cross ref.]*, the General Introduction provides guidance as follows (see document TG/1/3):
 - 5.3.3.2.1 Qualitative characteristics: "In qualitative characteristics, the difference between two varieties may be considered clear if one or more characteristics have expressions that fall into two different states in the Test Guidelines. Varieties should not be considered distinct for a qualitative characteristic if they have the same state of expression."
- 5.2.3.4 Thus, varieties which have different states of expression, i.e. different Notes, for the same qualitative characteristic can be considered to be distinct. Conversely, varieties which have the same Note for a qualitative characteristic should not be considered to be distinct for that characteristic.

Pseudo-qualitative (PQ) characteristics

5.2.3.5 The General Introduction (Chapter 5.3.3.2.3) states that "A different state in the Test Guidelines may not be sufficient to establish distinctness [...]. However, in certain

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circumstances, varieties described by the same state of expression may be clearly distinguishable.".

- 5.2.3.6 The number of Notes which may establish distinctness is influenced by factors such as location, year and environmental variation within the trial. Also, as with quantitative characteristics, the range of the scale (number of Notes) also varies. However, an important additional factor with pseudo-qualitative characteristics is that, whilst a part of the range is continuous, there is not an even distribution across the scale and it varies in more than one dimension (e.g. shape: ovate (1), elliptic (2), circular (3), obovate (4)). This means that it is difficult to define a general rule on the number of Notes to establish distinctness within a characteristic.
- 5.2.3.7 The following examples illustrate why deciding on the number of Notes required to establish distinctness needs particular care:

Example 1:

Type of mottling: only diffuse (1); diffuse and in patches (2); diffuse, in patches and linear bands (3); diffuse and in linear bands (4).

Example 2:

Shape: broad elliptic (1), medium elliptic (2), narrow elliptic (3), ovate (4)

Example 3:

Color: green (1), yellow green (2), green yellow (3), yellow (4), orange (5), red (6)

In the case of Examples 1 and 2, it is not appropriate to say that the "difference" between varieties with states 1 and 2 is less than between varieties with states 1 and 4, although they are respectively 1 and 3 Notes "different". In some cases, for example, the difference between Notes 2 and 3 may be greater than between Notes 1 and 4. However, Example 3 demonstrates that, for some pseudo-qualitative characteristics, it might be possible to follow a similar approach to that used for quantitative characteristics in some parts of the range e.g. varieties with states 2 and 3 (1 Note difference) have less difference than those with states 1 and 4 (3 Notes difference).

5.2.3.8 As explained in Section 4.1.4 (a) *[cross ref.]*, pseudo-qualitative characteristics are, in general, observed visually and are, therefore, usually recorded in the form of Notes. A particular exception to that is the case of color which is often recorded in the form of a color chart reference. Guidance on the use of color characteristics is provided in document TGP/14 Section 2.3: Glossary of Technical, Botanical and Statistical Terms Used in UPOV Documents: Botanical Terms: Color: color characteristics *[cross ref.]*.

Quantitative (QN) characteristics (vegetatively propagated and self-pollinated varieties)

5.2.3.9 The General Introduction states that:

"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."

- 5.2.3.10 Thus, it is the intention that the states and Notes are useful for the assessment of distinctness. It is recalled that this section considers the assessment of distinctness based on the information obtained from the growing trial and, therefore, refers to a situation where the states of expression and Notes are obtained for all varieties from the same growing trial in the same year. That situation is, in particular, reflected when the General Introduction states that:
 - "5.4.3 For quantitative characteristics, a difference of two Notes often represents a clear difference, but that is not an absolute standard for assessment of distinctness. Depending on factors, such as the testing place, the year, environmental variation or range of expression in the variety collection, a clear difference may be more or less than two Notes. Guidance is provided in document TGP/9, "Examining Distinctness."
- 5.2.3.11 Document TGP/7/1 "Development of Test Guidelines" (see Annex III: GN 20) explains that, in the case of quantitative characteristics, it is necessary to determine the appropriate range to describe the characteristic. In general, a standard "1-9" scale is used, but a "limited" range (Notes 1-5) and a "condensed" range (Notes 1-3) have also been accepted. Thus, when deciding on the number of Notes required to establish distinctness, the range of the scale will need to be taken into account.
- 5.2.3.12 In deciding, whether the "two-Note" standard is an appropriate basis for distinctness it is also necessary to take into account the environmental variation within the growing trial.
- 5.2.3.13 It should also be recalled that a pair of varieties which are not distinct for a characteristic on the basis of Notes may, for example in a subsequent growing trial, be directly compared, side-by-side, for a visual assessment of distinctness, where it may be possible to establish distinctness even where the two varieties are attributed the same Note for a quantitative characteristic.
- 5.2.3.14 In the case of single variety records other than Notes, no general guidance can be made and the size of the difference required for distinctness will, as for Notes, depend on factors such as the testing place, the year, environmental variation and the range of expression in the variety collection.

5.2.4 Statistical analysis of growing trial data

5.2.4.1 Where appropriate, the assessment of distinctness can be based on a statistical analysis of the data obtained from the growing trial. This approach requires that there is a

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sufficient number of records for a variety, e.g. a number of single, individual plants or parts of plants, whether obtained by measurement or by visual observation (MS / VS). In most cases, when a single record is obtained by visual observation or measurement of a group of plants (VG / MG), this results in a single record per variety, in which case it is not possible or necessary to apply statistical methods for the assessment of distinctness. However, in some cases, e.g. where there are several repetitions or plots, or more than one growing trial, more than one record per variety may be obtained, in which case statistical methods can be applied, although it is particularly relevant to check if the data obtained meets the assumptions required for a statistical procedure to be applied.

- 5.2.4.2 Visual assessment of distinctness is generally quicker and cheaper than the use of statistical analysis. However, as explained above, both visual assessment and the assessment of distinctness on the basis of Notes, require that the expression of the characteristic for a variety can be represented by a single record. That requirement implies that there should be very little variation within varieties, which is usually met for all characteristics of vegetatively propagated varieties and self-pollinated varieties and for qualitative and pseudo-qualitative characteristics for cross-pollinated and hybrid varieties, except in cases of segregating characteristics. Thus, the most common use of statistical analysis of growing trial data is for quantitative characteristics of cross-pollinated and some hybrid varieties.
- 5.2.4.3 The General Introduction makes the following recommendations with regard to the use of statistical methods in the assessment of distinctness:
 - "5.5 Interpretation of Observations for the Assessment of Distinctness with the Application of Statistical Methods

5.5.1 General

- 5.5.1.1 For measured characteristics as well as for visually assessed characteristics statistical methods can be applied. Appropriate methods have to be chosen for the interpretation of observations. The data structure and the type of scale from a statistical point of view (nominal, ordinal, interval or ratio) is decisive for the choice of appropriate methods. The data structure depends on the method of assessment (visual assessment) or measurements, observation of plots or single plants) which is influenced by the type of characteristic, the features of propagation of the variety, the experimental design and other factors. DUS examiners should be aware of certain basic rules of statistics and especially the fact that their use is linked to mathematical assumptions and the use of experimental design practices, such as randomization. Therefore, those assumptions should be verified before applying statistical methods. Some statistical methods are quite robust, however, and can be used, with some caution, even if some assumptions are not fully met.
- 5.5.1.2 Document TGP/8, "Use of Statistical Procedures in DUS Testing," provides guidance on some appropriate statistical procedures for DUS assessment and includes keys for the choice of methods in relation to the data structure.
- 5.5.1.3 A combined characteristic should only be used for distinctness if the uniformity criteria for the combined characteristic itself, and not only its components, have been satisfied.

5.5.2 <u>Visually Assessed^[*] Characteristics</u>

Non-parametric statistics may be used when visually assessed^[*] characteristics have been recorded on a scale that does not fulfill the assumptions of the usual parametric statistics. The calculation of the mean value, for example, is only permitted if the Notes are taken on a graded scale which shows equal intervals throughout the scale. In the case of non-parametric procedures, the use of a scale that has been established on the basis of example varieties representative of the different states of the characteristics is recommended. The same variety should then always receive about the same Note and thereby facilitate the interpretation of data. More details on the handling of visually assessed^[*] characteristics are given in document TGP/9, "Examining Distinctness."

- ([*] the term "observed" would be more consistent with the use of the terms "observed" and "assessed" in TGP/9)
- 5.2.4.4 The situations where statistical analysis might be used and the requirement for their use are summarized below:

Qualitative (QL) characteristics

5.2.4.5 The usual situation is that qualitative characteristics can be visually assessed and the General Introduction (Chapter 5.5.2.1) clarifies that "For visually assessed qualitative characteristics, different states of expression in direct comparisons are generally sufficient to assess distinctness. In most cases, therefore, no statistical methods are needed for the interpretation of the results."

Pseudo-qualitative (PQ) characteristics

5.2.4.6 The General Introduction (Chapter 5.5.2.3) explains that "The use of statistics for the assessment of pseudo-qualitative characteristics depends on the individual case, and no general recommendation can be made."

Quantitative (QN) characteristics (vegetatively propagated and self-pollinated varieties)

- 5.2.4.7 As indicated above, both visual assessment and the assessment of distinctness on the basis of Notes, are used for the assessment of distinctness for quantitative characteristics of vegetatively propagated and self-pollinated varieties. However, statistical methods can also be used for such situations where the necessary requirements are met.
- 5.2.4.8 The General Introduction clarifies the situation with regard to all visually observed, quantitative characteristics as follows:
 - 5.5.2 Visually Assessed^[*] Characteristics

[...]

5.5.2.2 Quantitative Characteristics

[...]

5.5.2.2.2 A direct comparison between two similar varieties is always recommended, since direct pairwise comparisons are the most reliable. In each comparison, a

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difference between two varieties is acceptable as soon as it can be assessed visually and could be measured, although such measurement might be impractical or require unreasonable effort.

- 5.5.2.2.3 The simplest case for establishing distinctness is when clear differences between varieties, in pair-wise comparisons, are of the same sign, provided these differences can be expected to recur in subsequent trials (e.g. variety A is consistently and sufficiently greater than B) and there are a sufficient number of comparisons. However, in most cases, establishing confidence that varieties are clearly distinguishable, is more complex. This is explained further in document TGP/9, "Examining Distinctness."
- 5.2.4.9 The situation referred to in the General Introduction that "However, in most cases, establishing confidence that varieties are clearly distinguishable, is more complex." does not, in general, apply to vegetatively propagated and self-pollinated varieties, but rather to the situation in cross-pollinated varieties and hybrid varieties. However, in most cases, quantitative characteristics are measured for such varieties and are handled as explained in Section 5.2.4.12 [cross ref.].
- 5.2.4.10 The General Introduction clarifies the situation with regard to measured, quantitative characteristics for vegetatively propagated and self-pollinated varieties as follows:

"5.5.3 <u>Measured Characteristics</u>

The following paragraphs provide guidance on the typical methods for examining distinctness according to the particular features of propagation of the variety:

[...]

5.5.3.1 Self-Pollinated and Vegetatively Propagated Varieties

UPOV has endorsed several statistical methods for the handling of measured quantitative characteristics. One method established for self-pollinated and vegetatively propagated varieties is that varieties can be considered clearly distinguishable if the difference between two varieties equals or exceeds the Least Significant Difference (LSD) at a specified probability level with the same sign over an appropriate period, even if they are described by the same state of expression. This is a relatively simple method but is considered appropriate for self-pollinated and vegetatively propagated varieties because the level of variation within such varieties is relatively low. Further details are provided in document TGP/9, "Examining Distinctness.""

([*] the term "observed" would be more consistent with the use of the terms "observed" and "assessed" in TGP/9)

5.2.4.11 Information on the Least Significant Difference (LSD) method is provided in TGP/8 *[cross ref.]*.

Quantitative (QN) characteristics (cross-pollinated varieties)

5.2.4.12 The General Introduction provides the following guidance with regard to the use of statistical methods for measured characteristics where individual plant data (MS) are available:

"5.5.3 Measured Characteristics

The following paragraphs provide guidance on the typical methods for examining distinctness according to the particular features of propagation of the variety:

[...]

5.5.3.2 Cross-Pollinated Varieties

5.5.3.2.1 COYD

UPOV has developed a method known as the Combined Over Years Distinctness (COYD) analysis, which takes into account variations between years. Its main use is for cross-pollinated, including synthetic, varieties but, if desired, it can also be used for self-pollinated and vegetatively propagated varieties in certain circumstances. This method requires the size of the differences to be sufficiently consistent over the years and takes into account the variation between years. It is explained further in document TGP/9, "Examining Distinctness."

5.5.3.2.2 Refined COYD

A refinement to the COYD analysis, which is also provided, should be used to adjust the COYD analysis when environmental conditions cause a significant change in the spacing between variety means in a year, such as when a late spring causes the convergence of heading dates. It is supplemented by a further LSD method for cases where few varieties in the growing tests lead to less than about 20 degrees of freedom for the estimation of standard error.

5.5.3.2.3 Non-Parametric Procedures

Where COYD analysis cannot be used because the statistical criteria are not fulfilled, non-parametric procedures can be considered.

5.2.4.13 The COYD method and the 2x1% criterion are explained in the following sections.

Combined Over-Years Distinctness Criterion (COYD)

5.2.4.14 To assess distinctness for varieties on the basis of a quantitative characteristic it is possible to calculate a minimum distance between varieties such that, when the distance calculated between a pair of varieties is greater than this minimum distance, they may be considered as "distinct" in respect of that characteristic. Amongst the possible ways of establishing minimum distances is the method known as the Combined-Over-Years Distinctness (COYD).

5.2.4.15 The COYD method involves:

- for each characteristic, taking the variety means from the two or three years of trials for candidates and established varieties and producing over-year means for the varieties;
- calculating a least significant difference (LSD), based on variety-by-years variation, for comparing variety means;
- if the over-years mean difference between two varieties is greater than or equal to the LSD then the varieties are said to be distinct in respect of that characteristic.

5.2.4.16 The main advantages of the COYD method are:

- it combines information from several seasons into a single criterion (the "COYD criterion") in a simple and straightforward way;
- it ensures that judgements about distinctness will be reproducible in other seasons; in other words, the same genetic material should give similar results, within reasonable limits, from season to season;
- the risks of making a wrong judgement about distinctness are constant for all characteristics.
- 5.2.4.17 Details on the use of the Combined-Over-Years Distinctness (COYD) are provided in document TGP/8 *[cross ref.]*.

2x1% criterion

- 5.2.4.18 For two varieties to be distinct using the 2x1% criterion, the varieties need to be significantly different in the same direction at the 1% level in at least two out of three years in one or more measured characteristics. The tests in each year are based on Student's two-tailed t-test of the variety means with standard errors estimated using the plot residual mean square from the analysis of the variety x replicate plot means.
- 5.2.4.19 With respect to the 2x1% criterion, compared to COYD, it is important to note that:
 - Information is lost because the criterion is based on the accumulated decisions arising from the results of t-tests made in each of the test years. Thus, a difference which is not quite significant at the 1% level contributes no more to the separation of a variety pair than a zero difference or a difference in the opposite direction. For example, three differences in the same direction, one of which is significant at the 1% level and the others at the 5% level would not be regarded as distinct.
 - Variety measurements on some characteristics are less consistent over years than on others. However, beyond requiring differences to be in the same direction in order to count towards distinctness, the 2x1% criterion takes no account of consistency in the size of the differences from year to year.

Other statistical methods

5.2.4.20 [There are a range of other statistical methods in use in agricultural research that can be used in the examination of distinctness. Those include ANOVA and multiple range tests. Providing the underlying assumptions are met, those other statistical methods are as acceptable as the other methods mentioned in this section.]^d

5.3 Summary of approaches for assessing distinctness based on the growing trial

The following table summarizes the common approaches to assessing distinctness based on the growing trial taking into account the method of propagation, type of expression of the characteristic, method of observation and the type of record.

	Type of expression of characteristic			
Method of propagation of the variety	QL	PQ	QN	
Vegetatively propagated, self-pollinated	Visual (V)	Visual (V)	Visual (V) Notes (VG/MG) Statistics (MG/MS)	
Cross-pollinated	Visual (V) Statistics (VS*)	Visual (V) Statistics (VS*)	Statistics (MG/MS/VS) (Visual (V)) (Notes (VG/MG))	
Hybrids	Visual (V) Statistics (VS*)	Visual (V) Statistics (VS*)	**	

^{*} records of individual plants only necessary if segregation is to be recorded

^{**} to be considered according to the type of hybrid (see section 4.3.3)

5.4 Techniques for assessing distinctness based on the growing trial

5.4.1 Parent Formula of Hybrid Varieties

In the case of variety collections which contain hybrid varieties it may be appropriate to use the parent formula as a basis for assessing distinctness based on the growing trial. The use of the parental formula requires that the difference between parent lines is sufficient to ensure that the hybrid obtained from those parents is distinct. Details of the parent formula technique are provided in TGP/8 *[cross ref.]*.

5.4.2 [Composite] / [Combined] / [Global] Phenotypic Distance

5.4.2.1 Introduction

[to be provided by experts from France based on document TWA/34/7 and the comments made by the Technical Working Party for Agricultural Crops (TWA) at its thirty-fourth session (see document TWA/34/14, paragraph 40)]

- 5.4.2.2 *Methods*
- 5.4.2.2.1 GAIA
- 5.4.2.2.1.1 The GAIA method, developed by experts from France, calculates a phenotypic distance between two varieties, which is a sum of distances for individual characteristics. For each genus or species, this system is calibrated to determine: (a) the weighting given to differences in each characteristic; and (b) the threshold, called "Distinctness plus" threshold, for the phenotypic distance used to determine distinctness eliminate varieties from the growing trial. The experience of crop experts is necessary to calibrate different parameters.
- 5.4.2.2.1.2 The GAIA software has been developed to automate these comparisons. Details of the GAIA method are provided in TGP/8 *[cross ref.]*.

5.5 Illustrative scenarios

5.5.1 Vegetatively propagated / Self-pollinated varieties: Small trial

Type of varieties: vegetatively propagated / self-pollinated

Grouping efficiency: high Size of growing trial: small

Trial design: direct side-by-side comparisons

Number of growing cycles: one

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	QL	PQ	QN
Type of observation	Visual	Visual	Visual
Type of record for distinctness assessment	-	-	-
Distinctness assessment	Visual	Visual	Visual
Techniques	-	-	-
Type of record for description in variety collection	Notes / single variety records	Notes / single variety records	Notes / single variety records

5.5.2 Self-pollinated varieties: Large trial (non-replicated)

Type of varieties: self-pollinated

Grouping efficiency: low Size of growing trial: large

Trial design: grouping of similar varieties (non-randomized)

Number of growing cycles: two

	Number of growing cycles. two		
	QL	PQ	QN
Type of observation	Visual	Visual	Visual / Measurement
Type of record for distinctness assessment	VG	VG	VG/MG
Primary distinctness assessment	Notes / single variety records	Notes / single variety records	Notes / single variety records
Distinctness assessment techniques	[Composite] / [Combined] / [Global] Phenotypic Distance		
Secondary distinctness assessment (if required)	-	Visual (side-by-side)	Visual (side-by-side)
Type of record for description in variety collection	Notes / single variety records	Notes / single variety records	Notes / single variety records

5.5.3 Self-pollinated varieties: Large trial (replicated plots)

Type of varieties: self-pollinated Grouping efficiency: low Size of growing trial: large Trial design: replicated plots Number of growing cycles: two

	QL	PQ	QN
Type of observation	Visual	Visual	Visual / Measurement
Type of record for distinctness assessment	VG	VG	VG/MG (replicated)
Primary distinctness assessment	Notes / single variety records	Notes / single variety records	Statistical analysis
Distinctness assessment techniques	-	-	statistical method
Secondary distinctness assessment (if required)	-	Visual (side-by-side)	Visual (side-by-side)
Type of record for description in variety collection	Notes / single variety records	Notes / single variety records	Notes / single variety records

5.5.4 Cross-pollinated varieties

Type of varieties: cross-pollinated Grouping efficiency: low

Size of growing trial: large
Trial design: randomized trial
Number of growing cycles: two

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	QL	PQ	QN
Type of observation	Visual	Visual	Visual /Measurement
Type of record for distinctness assessment	VG	VG	VS/MS/MG
Distinctness assessment	Notes / single variety records	Notes / single variety records	Statistical analysis
Distinctness assessment techniques	-	-	COYD
Type of record for description in variety collection	Notes / single variety records	Notes / single variety records	Notes / single variety records or data (cyclical planting)

5.5.5 Hybrids

Type of varieties: hybrids
Grouping efficiency: low
Size of growing trial: large
Trial design: grouping of similar varieties (non-randomized)

	QL	PQ	QN	
Type of observation	Visual	Visual	Visual / Measurement	
Type of record for distinctness assessment	VG	VG	VG/MG	
Primary distinctness assessment	Notes / single variety records	Notes / single variety records	Notes / single variety records	
Distinctness assessment	[Composite] / [Combined] / [Global] Phenotypic Distance			
techniques	Parent formula			
Secondary distinctness assessment (if required)	-	Visual (side-by-side)	Visual (side-by-side)	
Type of record for description in variety collection	Notes / single variety records	Notes / single variety records	Notes / single variety records	

SECTION 6: SUPPLEMENTARY PROCEDURES

6.1 Introduction

The General Introduction explains that:

5.3.1.2 In addition, certain supplementary procedures may be developed to avoid the need for a systematic individual comparison. For example, the publication of variety descriptions, inviting comment from interested parties, or cooperation between members of the Union, in the form of an exchange of technical information, could be considered as supplementary procedures. However, such an approach would only be possible where the supplementary procedures, in conjunction with the other procedures, provide an effective examination of distinctness overall. Such procedures may also be appropriate for consideration of varieties of common knowledge, for which living plant material is known to exist (see section 5.2.2) but where, for practical reasons, material is not readily accessible for examination. Any such procedures are set out in document TGP/9, "Examining Distinctness."

6.2 Publication of variety descriptions

The General Introduction notes that the publication of variety descriptions inviting comment from interested parties may be considered as a supplementary procedure to avoid the need for a systematic individual comparison (see document TG/1/3, section 5.3.1.2). An example of the use of such a procedure can be found in document TGP/6 Section 2.2, which explains the procedure used in Australia.

6.3 Cooperation between members of the Union

The General Introduction states that cooperation between members of the Union in the form of exchange of technical information could also be used as a supplementary procedure (see document TG/1/3, section 5.3.1.2).

6.4 Use of randomized "blind" testing

- 6.4.1 After, or during, the examination, some doubts may exist over the distinctness of a variety on the basis of the trials. In such cases, the following situations are possible:
 - (a) with no differences observed, the application is rejected;
 - (b) with no conclusive difference observed and a claim from the applicant, the examining authority may decide to arrange additional tests.
- 6.4.2 In the case of visually observed characteristics one possible arrangement for the additional test is "blind" testing.

- 6.4.3 The aim of "blind" testing is to assess distinctness between a pair of varieties avoiding any pre-judgement in the observation by making the samples in the trial anonymous (the expert is "blind" in respect to the identity of the variety in each plot). This kind of test plays a clarifying role when the differences between the candidate and (a) similar variety(ies) are not clearly definable. In such a case, another test during or after the examination of distinctness may provide evidence for a definitive decision by the authority.
- 6.4.4 The following are some examples of "blind" testing:

Randomized variety plots: duplicates samples of the same variety receive individual codes and are randomly distributed in the trial.

Plots containing a mixture of varieties: plots with a mixture of material from the varieties under examination are included in the trial. [This can be useful for seed propagated varieties].

Parts of plants of varieties: randomized parts of plants from the varieties under examination (e.g. leaves or fruit).

- 6.4.5 Applicants may be part of the "blind" testing process. They may also be invited to visit the "blind" test and be requested to try to identify the plots of their variety.
- 6.4.6 At the end of the "blind" testing, a variety may be declared as distinct:
 - (a) if the expert and, where appropriate, the applicant always identify the variety; and
 - (b) the difference can be considered as a clear difference for that characteristic.
- 6.4.7 In all cases, it is the authority which decides on distinctness.

6.5 The advice of plant experts

There may be cases where the assistance of a recognized plant expert or group of plant experts with extensive knowledge of varieties of common knowledge in a given genus, species or type of variety may be appropriate. In these cases, it is recommended that clear rules on the tasks and responsibilities of the plant expert or group of plant experts involved, as well as on the management of the information submitted for the purposes of examination, be established in order to maintain the transparency of the system.

[End of document]

^a the TWF agreed that consideration should be given to adding "The most important consideration in selecting varieties for inclusion in the growing trial is the identification of the most similar varieties of common knowledge. Once identified, at least the most similar varieties should be included in the variety collection and the growing trial. Other similar varieties may be

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excluded on the basis of grouping characteristics". The TWA urged caution at the use of the term "most similar varieties" in a way which could cause confusion with the use of the term "similar variety" elsewhere and, in particular, its use in Section 6 of the Technical Questionnaire and in the UPOV Variety Description (see document TGP/5 "Experience and Cooperation in DUS Testing" Section 6: 16)

^b the TWF agreed that consideration should be given to adding the text shown

^c the TWV noted that the definition of the terms differs from those used in TGP/7 and suggested to consider any consequences of this changed definition for TGP/7. The TWV noted that the new definitions and explanations were very clear, but clarified that the terms were only concerned with the form of the data produced and did not provide any guidance to examiners on whether, for example, an MG observation should involve the observation of several individual plants or could be done by a single global assessment of the plot. It agreed that such advice was important in the context of the Test Guidelines, which were aimed at DUS examiners. The TWF proposed to retain the indication of whether a characteristic should be observed visually (V) or measured (M), but not to include any indication of whether the observation should be made on single, individual plants or on groups of plants. It was noted that any reference to individual plants, if retained, should also make reference to parts of plants. The TWO noted that an indication of whether a characteristic should be visually observed or measured might be useful in some circumstances, but did not consider that it should be obligatory in all Test Guidelines. It considered that an indication of whether observations should be made on individual plants or groups of plants and whether a single record or multiple records should be kept would be inappropriate for Test Guidelines covering ornamental plants.

d the TWF agreed that consideration should be given to adding the text shown