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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

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/10

"EXAMINING UNIFORMITY"

Document prepared by the Office of the Union

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SECTION 1: INTRODUCTION

1.1 According to Article 6(1)(c) of the 1961/1972 and 1978 Acts of the UPOV Convention, a variety is deemed uniform if it is "sufficiently homogeneous, having regard to the particular features of its sexual reproduction or vegetative propagation." Article 8 of the 1991 Act deems that a variety is uniform if, "subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics".

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", Chapter 6.2, clarifies that "Relevant characteristics of a variety include at least all characteristics used for the examination of DUS or included in the variety description established at the date of grant of protection of that variety. Therefore, any obvious characteristic may be considered relevant, irrespective of whether it appears in the Test Guidelines or not". ~~Hence, it is a matter for the authority to decide, in addition to those characteristics included in the UPOV Test Guidelines or national guidelines, which other characteristics it may include in its consideration of uniformity.~~[a] [b]

1.3 This document explains how the variation in the expression of relevant characteristics within varieties is used as the basis for the assessment of uniformity, and provides an overview of the two main approaches to the assessment of uniformity; namely off-types and standard deviations. Details on some of the techniques used in those approaches are provided in TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability" (document TGP/8) *[cross ref.]*[c] and cross references are made in the appropriate sections of this document.

SECTION 2: VARIATION IN THE EXPRESSION OF CHARACTERISTICS WITHIN VARIETIES

2.1 Introduction

The variation in the expression of relevant characteristics within varieties is the basis for the assessment of uniformity. This variation [is always present to some extent and][d] has both genetic components and environmental components (e.g. temperature, light, soil etc.).[e] The level of variation due to the environment depends on the interaction between individual plants and the environment and is influenced by the type of expression of the characteristic. The genetic component is mainly influenced by the features of propagation.

2.2 Type of expression of the characteristic

For quantitative and pseudo-qualitative characteristics, the level of variation due to the environment can differ from species to species and from characteristic to characteristic. There is usually little environmental variation for qualitative characteristics.[f]

2.3 Features of propagation of the variety

2.3.1 With regard to genetic variation and the particular features of propagation of a variety:

(a) a low level of genetic variation is expected for vegetatively propagated (e.g. apricot, avocado)[g] and truly self-pollinated (e.g. rice, soybean, wheat)[h] varieties. Variation in the expression of characteristics within such varieties should result, predominantly, from environmental influences;

(b) variation in the expression of characteristics within mainly self-pollinated varieties (e.g. cotton, triticale)[h] should also result, predominantly, from environmental influences but a low level of genetic variation caused by some cross pollination is accepted. Therefore, more variation may be tolerated than for vegetatively propagated and truly self-pollinated varieties;

(c) in cross-pollinated varieties (including synthetic varieties), variation in the expression of characteristics within varieties results from both genetic and environmental components.[i] The overall level of variation is, therefore, generally higher in cross-pollinated and synthetic varieties. In relation to self-pollinated, vegetatively propagated and mainly self-pollinated varieties a higher genetic variation is accepted[j];

(d) genetic variation in hybrid varieties depends on the type of hybrid (single- or multiple-cross), the level of genetic variation in the parental lines (inbred lines or others) and the system for hybrid seed production (mechanical emasculation, system of male sterility etc.). The tolerance limits for uniformity of hybrid varieties[k] are set according to the specific situation resulting from genetic and environmental influences on the variation in the expression of characteristics.

2.3.2 As noted in Section 1 *[cross ref.]*, the UPOV Convention requires consideration of the uniformity of a variety on the basis of "… the variation that may be expected from the particular features of its propagation, …". Thus, the General Introduction, Chapter 6.4, explains "Where all the plants of a variety are very similar, and in particular for vegetatively

propagated and self-pollinated varieties, it is possible to assess uniformity by the number of obviously different plants – "off-types" – that occur. However, where the range[l] of variation within a variety is larger, because of the features of its propagation, and in particular for cross-pollinated, including synthetic, varieties, the plants are not all very similar and it is not possible to visualize which plants should be considered as atypical or "off-types." In this case the uniformity can be assessed by considering the overall range[l] of variation, observed across all the individual plants, to determine whether it is similar to comparable varieties".

2.3.3 The assessment of uniformity by the off-type approach and by consideration of the overall range[l] of variation ("standard deviations approach") is set out in Sections 4 and 5, respectively.

2.4 Segregating characteristics

2.4.1 The General Introduction, Chapter 6.4.3.4.1, explains that "For other than single-cross hybrids (e.g. three-way crosses or double crosses), a segregation of certain characteristics is acceptable if it is compatible with the method of propagation of the variety. Therefore, if the heredity of a clear-cut segregating characteristic is known, it is required to behave in the predicted manner. If the heredity of the characteristic is not known, it is treated in the same way as other characteristics in cross-pollinated varieties, i.e. relative tolerance limits, for the range[l] of variation, are set by comparison with comparable varieties, or types, already known […]". In addition, for synthetic varieties, a segregation of certain characteristics is acceptable if it is compatible with the method of propagation of the variety.

2.4.2 Thus, for multiple-cross hybrids and synthetic varieties, a segregation for certain characteristics, in particular for qualitative characteristics, is accepted if it is compatible with the expression of the parental lines and the method of propagating the variety. If the inheritance of a segregating characteristic is known, the variety is considered to be uniform if the characteristic behaves in the predicted manner. This can be determined by using a standard statistical procedure such as the χ^2 test.[m] [(see document TGP/8)][d]

2.4.3 If the inheritance of a clear-cut segregating characteristic is not known, the observed segregation ratio should be described.

2.4.4 In quantitative characteristics, segregation in multiple-cross hybrids and synthetic varieties may result in a continuous variation. In such cases, uniformity is assessed as in cross-pollinated varieties, on the basis of standard deviations.

2.5 Summary

2.5.1 The type of variation in the expression of a characteristic within a variety determines how that characteristic is used to determine uniformity in the crop. In cases where it is possible to "visualize" off-types, the off-type approach is recommended for the assessment of uniformity. In other cases, the standard deviations approach is used. Thus, the uniformity of a variety may be determined by off-types alone, by standard deviations alone, or by off-types for some characteristics and by standard deviations for other characteristics.

2.5.2 The following table summarizes the common approaches for the assessment of uniformity, taking into account the method of propagation, type of expression of the characteristic and the method of observation. The most common approaches are listed first.[n]

	Type of expression of characteristic		
Method of propagation of the variety	QL	PQ	QN
Vegetatively propagated	*Off-types*	*Off-types*	*Off-types (visual observation)* *Standard Deviations (measurement)[o]*
Self-pollinated	*Off-types*	*Off-types*	*Off-types (visual observation)* *Standard Deviations (measurement)[o]*
Cross-pollinated	*Off-types*	*Off-types*	*Standard Deviations*
Single-cross hybrid (in-bred parent lines)	*Off-types*	*Off-types*	*Off-types (visual observation)* *Standard Deviations (measurement)[o]*
Other hybrids	*	*	*

* To be considered according to the type of hybrid.

SECTION 3: METHOD OF OBSERVATION OF CHARACTERISTICS[p]

3.1 Off-type approach

As with the observation of characteristics for distinctness (see document TGP/9 "Examining Distinctness" (document TGP/9), Section 4.2 *[cross ref.]*), qualitative and pseudo-qualitative characteristics are, in general, observed visually and off-types are determined by visual assessment. For vegetatively propagated and self-pollinated varieties there is very little[q] variation within varieties and, as with the observation of characteristics for distinctness for such varieties, quantitative characteristics are commonly observed visually, with off-types being determined by visual assessment. In some cases, measurements may be taken from individual plants in order to assess off-types for quantitative characteristics. The use of visual observation and measurements for determining off-types is considered in Section 4 *[cross ref.]*.

3.2 Standard deviations approach

3.2.1 As with the observation of characteristics for distinctness (see document TGP/9, Section 4.2 *[cross ref.]*), qualitative and pseudo-qualitative characteristics are, in general, observed visually.

3.2.2 In the case of the standard deviations approach, the choice of visual observation or measurements for quantitative characteristics, may take into account the following factors:

(a) visual observations are generally quicker and cheaper than measurements but, because they are based on the expert's judgement, they have a particularly important 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; visual observations are appropriate if the [resultant][d] data fulfill the conditions for calculation of mean and standard deviation:

(b) measurements may be required in order to provide the appropriate precision for the assessment of variation

~~3.3 Combination of off-types and standard deviations~~ [r]

SECTION 4: UNIFORMITY ASSESSMENT ON THE BASIS OF OFF-TYPES[s]

4.1 Introduction

The General Introduction, Chapter 6.4, states that "Where all the plants of a variety are very similar, and in particular for vegetatively propagated and self-pollinated varieties, it is possible to assess uniformity by the number of obviously different plants – "off-types" – that occur". This section considers the use of the off-type approach. In general, off-types are observed visually, although this section also considers the possibility of off-types being determined on the basis of measurements.

4.2 Plants which are not considered as Off-Types [t]

4.2.1 Atypical plants which are not considered to be Off-types

4.2.1.1 It is important to differentiate between genetic causes of atypical expression in plants or parts of plants, such as mutation and cross-pollination, and external factors such as environment, disease and cultural practice. Where the atypical expression of a plant or a part of the plant does not have a genetic basis, the plant should not be considered to be an off-type. Examples of[u] external factors which may cause atypical expression include:

(a) positional effects:
  – exposure to different levels of light or temperature (e.g. due to different positions in the plot) can produce different colors, different levels of anthocyanin, or different levels of variegation;
  – variations in fertility, pH or moisture across the plot or, in the case of pot-grown plants, between pots;
(b) infection by disease;
(c) pest infestation;
(d) graft incompatibility (example: Graft incompatibility in *Gymnocalycium mihanovichii* (Chin Cactus) can change the color of the scion);
(e) conditions or treatments experienced by plant material prior to supply for testing, e.g. quarantine requirements, in vitro propagation.[v]

4.2.1.2 The General Introduction, Chapter 6.5, explains that "The test material may contain plants that are very atypical or unrelated to those of the variety. These are not necessarily treated as off-types, or part of the variety, and may be disregarded, and the test may be continued, as long as the removal of these very atypical or unrelated plants does not result in an insufficient number of suitable plants for the examination, or make the examination impractical. In choosing the term 'may be disregarded,' UPOV makes it clear that it will depend on the judgment of the crop expert. In practice, in tests conducted with a small number of plants, just one single plant could interfere with the test, and therefore should not be disregarded.". For example, a plant that does not belong to the species of the candidate variety may be considered not to be an off-type and might be disregarded. In cases where the atypical plants are of the same species as the candidate variety it is more difficult to decide that the plants are very atypical or unrelated.

4.2.2 Within-plant variation which does not indicate an Off-type plant

4.2.2.1 It is important to recognize that variation within a plant may not be an indication of a lack of uniformity, particularly if the within-plant variation is consistent between plants.

[Within plant variation can be caused by an external influence (e.g. light levels of the inner and outer plant) or genetically based.][v] For example, in a zonal Pelargonium variety there may be variation in the number of white stripes on red florets. Within each plant there may be some flowers with almost no white stripes, some flowers with approximately half the surface area white and half red, and some flowers that have more white than red. Although the flowers in each plant do not have an identical color pattern, if the variation in striping is consistent in all plants, then the variety can be considered uniform. In the case of Regal Pelargonium, if non-fully purple petals are present on all plants at the same frequency, then this does not indicate a lack of uniformity. However, plants which have a significantly different frequency of non-fully purple petals may be off-types.

4.2.2.2 When assessing whole-plant characteristics, the expert should be careful not to focus on the individual plant parts. An example could be a variety with a prostrate growth habit, but where some of the shoots are erect in similar frequency on all plants. The shoots which are erect would not be considered as an indication of an off-type plant, provided the different expression did not have a genetic basis, for example as a result of a somaclonal mutation within the plant[w].

4.2.3 Further investigation [x]

Determining whether an atypical plant or within-plant variation should be considered to constitute an off-type plant may require further investigation (see Section 4.3.3 *[cross ref.]*).

4.3 Determination of Off-Types by Visual Assessment

4.3.1 Introduction

The General Introduction states the following with respect to the observation of characteristics for uniformity using the off-type procedure:

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

Thus, the following aspects are relevant for determining off-types:

(a) the standard for distinctness between a candidate variety and any other variety, taking into consideration the particular features of its propagation; and
(b) the expression of any characteristic of the whole or part of the plant used in the testing of distinctness;

4.3.2 Guidance for determining off-types[y]

4.3.2.1 The same principles used for the determination of distinctness between varieties should be applied to the determination of individual off-type plants within a variety for the assessment of uniformity. Thus, in order to identify any plant as an off-type plant, that plant

should be clearly distinguishable from the plants which form the variety, taking into consideration the particular features of its propagation. ~~That requirement means that an off-type plant could potentially become a distinct variety if it could be propagated unchanged~~.[b]

4.3.2.2 ~~Creating a definitive model for identifying off-types is not possible, considering the very large range of genera and species within which examination of Distinctness, Uniformity and Stability (DUS) is required.~~[b] The guidance in this document is intended to identify factors to be taken into account for the determination of off-types in order that there can be a harmonized approach. This guidance demonstrates the need for the DUS examiner to have a good level of experience within the genus or species concerned, or within a similar genus or species. [z]

4.3.2.3 In cases where it is evident that the atypical expression of a plant has a genetic basis and where the plant is clearly distinguishable from the plants which form the variety, taking into consideration the particular features of its propagation, it can be considered to be an off-type.

4.3.2.4 A difference in the expression of a characteristic may occur on one part of the plant, but not consistently throughout the plant. The genetic causes of such atypical expression include mutations, chimeras and transposons. It may be observed that one part of the plant might be atypical: for example, a single green shoot where all the other shoots are red, a single green shoot in a variegated variety, a part of the plant with spotting or flecking. The DUS examiner must decide in such cases whether, for example, a plant with one green shoot is an off-type. In that respect, atypical expression caused by genetic factors, such as mutation, on any part of the plant are very likely to lead to the whole plant being considered an off-type.[aa] ~~These considerations should be borne in mind when fixing the number of plants to be examined in the DUS trial. Small sample sizes which do not allow any off-types mean that the occurrence of any chance mutation may cause the rejection of the variety~~.[bb]

4.3.2.5 An off-type plant could be indicated by the nature, type and frequency of the variation in expression. Thus, in some cases, the simple presence or absence of atypical expression of a characteristic may be enough to indicate whether a plant is an off-type. In other cases, the presence or absence alone of atypical expression of a characteristic may not be sufficient and the frequency of the atypical expression may also require consideration. For example, if there were only one plant with a green shoot[cc] in a variegated variety, then that plant might be considered to be an off-type. However, if all plants had at least one green shoot, then that may be considered to be the typical expression of the variety. The situation becomes more difficult when, for example, most of the plants have a few green shoots, but some do not. [A second example can be seen in apple fruit coloration and patterning. The fruit color, color intensity, amount of overcolor and pattern of overcolor can have atypical expression present, but it is the frequency of the variation which requires consideration.] All plants of the variety in the trial must be able to be described in the same way according to the UPOV Test Guidelines. If this is not possible then the plants in trial do not form a uniform variety. [dd]

4.3.3 <u>Investigating plants with atypical expression</u>[ee]

4.3.3.1 In cases of doubt with regard to whether a plant is an off-type, in particular where the DUS examiner has limited experience with the genus or species, an important first step is to consult the breeder. ~~In some cases, for example, it may help the DUS examiner to visit the~~

~~breeder's premises in order to view a larger sample of plants~~.[ff] Consultation with other DUS examiners, panels of experts, botanists, botanical gardens, plant collectors etc. may also be helpful.

4.3.3.2 It is important to mark the plant or plant part which is atypical, so that the development of the plant/plant part can be observed over time. It can also be helpful to photograph the plant/plant part at suitable times, in particular where the expression is likely to have a short duration, e.g. characteristics concerning the flower.

4.3.3.3 In cases where there is still uncertainty at the end of a growing cycle about whether or not a plant is an off-type, in particular concerning the genetic basis or otherwise of atypical expression, the variety could be observed in a further growing cycle. [This can be carried out on the existing material for a second cycle or on new material and is not specifically intended as a test for stability.][v] Depending on the features of propagation of the variety, a further growing cycle may allow the atypical plant or part of the plant to be propagated and compared with typical plants of the variety. Depending on the circumstances, a new batch of typical plants might be requested from the breeder and/or a new generation of plants might be obtained from propagation of typical plants in the DUS trial. That would also allow measures to be taken concerning the phytosanitary status of the material, if that was considered to be a possible cause of the atypical expression.[gg]

4.4 Determination of Off-Types Using Measurements

4.4.1 The General Introduction states the following:

> "*6.4.1.2 Determination of Off-Types Using Measurements*
>
> Most characteristics of self-pollinated and vegetatively propagated varieties are observed visually, or by making a single measurement in a group of plants. However, where appropriate, methods of handling measurements from individual plants, in order to assess off-types in truly or mainly self-pollinated varieties and vegetatively propagated varieties, are set out in document TGP/10, "Examining Uniformity"."

4.4.2 [to be developed][hh]

~~Notwithstanding Chapter 6.4.1.2 of the General Introduction, it has not been considered appropriate to consider methods of handling measurements from individual plants, in order to assess off-types in truly or mainly self-pollinated varieties and vegetatively propagated varieties~~.

[section to be drafted by Mr. Niall Green]

4.5 Acceptable number of off-types

4.5.1 Self-Pollinated and Vegetatively Propagated Varieties

4.5.1.1 The General Introduction, Chapter 6.4.1.3, explains that "The acceptable number of off-types tolerated in samples of various sizes is often based on a fixed "population standard" and "acceptance probability". The "population standard" can be expressed as the maximum percentage of off-types to be accepted if all individuals of the variety could be examined. The

probability of correctly accepting as uniform a variety with the population standard of off-types is called the "acceptance probability".[ii]

4.5.1.2 As explained in Section 2 *[cross ref.]*, the off-type approach is the common method of assessing uniformity in self-pollinated and vegetatively propagated varieties. However, the General Introduction, Chapter 6.4.1.3.2, explains that "For the purpose of DUS testing, mainly self-pollinated varieties are those that are not fully self-pollinated but are treated as self-pollinated for testing. For these, as well as for inbred lines of hybrid varieties, a higher tolerance of off-types can be accepted, compared to truly self-pollinated and vegetatively propagated varieties […]". Nevertheless, where appropriate, the same tolerance may be used.[jj]

4.5.1.3[jj] An additional tolerance of off-types can be accepted for clear cases of out-crossed plants in inbred lines as well as plants obviously resulting from the selfing of a parent line in single-cross hybrids.

4.5.1.4 The UPOV Test Guidelines recommend for a particular type(s) of variety a general, i.e. "fixed", population standard and acceptance probability and provide the maximum[kk] acceptable number of off-types for an appropriate sample size. The population standard and acceptance probability, together with an appropriate sample size, are selected [ll] on the basis of experience, in particular with reference to other UPOV Test Guidelines for comparable types of variety.

4.5.1.5 In the absence of UPOV Test Guidelines, an appropriate population standard and acceptance probability, together with the maximum[kk] acceptable number of off-types for an appropriate sample size, are selected[k] on the basis of experience, in particular with reference to UPOV Test Guidelines for comparable types of variety.

4.5.1.6 Larger plant numbers may be appropriate for the assessment of varieties which are more likely to contain off-types (e.g. varieties resulting from mutation, containing transposons, variegated varieties etc.), in order to allow a suitable assessment of potential off-types. Some UPOV Test Guidelines for vegetatively propagated varieties recommend a population standard of 1% and an acceptance probability of at least 95 %, with 1 off-type plant permitted for a sample size of between 6-35 plants. A larger sample size could be selected from within the same range for the same number of off types. This provides the benefits of a larger sample without increasing the number of permitted off-types and, thereby, increasing the risk of accepting a non uniform variety. Small plant numbers which do not allow any off-types have the risk that the occurrence of any chance mutation may cause the rejection of the variety.[mm]

4.5.1.7 Detailed guidance on the use of the off-type approach, including tables of maximum acceptable[kk] numbers of off-types for given sample sizes corresponding to fixed population standards and acceptance probabilities, is provided in document TGP/8 *[cross ref.]*. [The sample size and maximum acceptable number of off-types must be selected with care in order to produce a good test.][nn]

4.5.2 Cross-pollinated Varieties

In some cases of cross-pollinated varieties, in particular for qualitative and pseudo-qualitative characteristics, the great majority of individuals of a variety may have very similar expression, such that plants with a clearly different expression can be detected as off-types

(e.g. "Root: color …" in fodder beet, "Root: color" in fodder radish). In such cases the off-type procedure is appropriate. The number of off-types of a candidate variety should not significantly exceed the number found in comparable varieties already known. Thus, the population standard should reflect the number of off-types found in comparable varieties.

4.6 Setting standards for new types and species [oo]

As explained in Section 4.5.1.5 *[cross ref.]*, in the absence of UPOV Test Guidelines, an appropriate population standard and acceptance probability, together with the maximum acceptable number of off-types for an appropriate sample size, are selected on the basis of experience, in particular with reference to UPOV Test Guidelines for comparable types of variety. Comparable types of variety may relate to varieties of a species belonging to the same genus, or may relate to varieties of a different genus. In that respect, it should be recalled that the uniformity requirement is based on the features of propagation of the variety and, therefore, comparable varieties should be those which have the most similar features of propagation (see Section 2.3 *[cross ref.]*). In particular, varieties of the same genus or species which have different features of propagation (e.g. vegetatively propagated varieties and cross-pollinated varieties) need to be considered separately with regard to uniformity standards. In the case of interspecific and intergeneric hybrids, the "parent" species and genera should, in particular, be considered with regard to comparable varieties. The breeder is likely to be an important source of information concerning the features of propagation of the variety and can provide information in the Technical Questionnaire or by other means concerning the breeding method used. (see also document TGP/13 "Guidance for New Types and Species" (document TGP/13))[kk].

SECTION 5: UNIFORMITY ASSESSMENT ON THE BASIS OF STANDARD DEVIATIONS

5.1 Introduction

The General Introduction, Chapter 6.4, explains that, in cases where there is a wide range[l] of variation in the expressions of characteristics for the plants within a variety, it is not possible to visualize which plants should be considered as off-types and the off-type approach for the assessment of uniformity is not appropriate. It clarifies that in such cases, uniformity can be assessed by considering the overall range[l] of variation, observed across all the individual plants, to determine whether it is similar to comparable varieties. In this approach, relative tolerance limits for the range[l] of variation are set by comparison with comparable varieties, or types, already known ("standard deviations approach"). The standard deviations approach means that a candidate variety should not be significantly less uniform than the comparable varieties.

5.2 Determining the acceptable level of variation

5.2.1 The comparison between a candidate variety and comparable varieties is carried out on the basis of standard deviations, calculated from individual plant observations. Comparable varieties are varieties of the same type within the same or a closely related species that have been previously examined and considered to be sufficiently uniform.[oo]

5.2.2 UPOV has proposed several statistical methods for dealing with uniformity in measured quantitative characteristics. One method, which takes into account variation between years, is the Combined Over Years Uniformity (COYU) method. The comparison between a candidate variety and comparable varieties is carried out on the basis of standard deviations, calculated from individual plant observations. This COYU procedure calculates a tolerance limit on the basis of comparable varieties already known i.e. uniformity is assessed using a relative tolerance limit based on varieties within the same trial with comparable expression of characteristics[pp].

5.2.3 Details of the COYU method are provided in document TGP/8 *[cross ref.]*.

5.2.4 ~~If the conditions for the application of the COYU procedure are not fulfilled e.g. the test is performed for only one year, or the number of tested varieties is too small, other appropriate statistical methods should be used for the comparison of standard deviations (e.g. 1.26 x standard deviations,[qq] 1.6 x variance, long term LSD).[rr]~~ Information on other appropriate statistical methods (e.g. 1.26 x standard deviations, 1.6 x variance, ~~long term LSD~~[ss]) is provided in document TGP/8 *[cross ref.]*

5.3 Setting standards for new types and species

As explained in Section 5.1 *[cross ref.]*, in cases where the off-type approach is not appropriate, relative tolerance limits for the range of variation are set by comparison with comparable varieties, or types, already known ("standard deviations approach"). The standard deviations approach means that a candidate variety should not be significantly less uniform than the comparable varieties. Comparable varieties may relate to varieties of a species belonging to the same genus, or may relate to varieties of a different [,but closely related,][o] genus. In that respect, it should be recalled that the uniformity requirement is based on the features of propagation of the variety and, therefore, comparable varieties should be

those which have the most similar features of propagation (see Section 2.3 *[cross ref.]*). In particular, varieties of the same genus or species which have different features of propagation (e.g. vegetatively propagated varieties and cross-pollinated varieties) need to be considered separately with regard to uniformity standards. In the case of interspecific and intergeneric hybrids, the "parent" species and genera should, in particular, be considered with regard to comparable varieties. The breeder is likely to be an important source of information concerning the features of propagation of the variety and can provide information in the Technical Questionnaire or by other means concerning the breeding method used.[oo] (see also document TGP/13)[kk]

SECTION 6: COMBINING OBSERVATIONS FOR ALL CHARACTERISTICS[tt]

6.1 Introduction

The uniformity of a variety is assessed by the observation of individual plants for all relevant characteristics. In some crops, all the characteristics are observed on all plants in the test. In other crops, different characteristics are observed on different samples of the variety. Furthermore, for some crops the assessment of uniformity may be on the basis of off-types for certain characteristics and on the basis of standard deviations for other characteristics. Therefore, specific rules for the assessment of uniformity based on the observation of all the relevant characteristics need to be defined. Some of the possible situations are described below:

6.2 Off-types only: all characteristics observed on the same sample

An off-type plant may be obviously different from the variety on the basis of one or several characteristics, but it will only be counted as one off-type plant, irrespective of the number of characteristics for which it has an obviously different expression. In cases where the assessment of uniformity is on the basis of off-types for all characteristics, and is by visual observation of all plants in the test, off-type plants can be marked as soon as an "off-type" expression is observed for at least one characteristic. It is not necessary to observe the off-type plant after that time. Additional off-type plants might be identified at a later stage of the test after the observation of further characteristics. The total number of off-types is determined after the observation of all relevant characteristics, and the uniformity of the variety is assessed by reference to the sample size and the population standard.

6.3 Off-types only: characteristics observed on different samples

In many cases, uniformity is assessed by observations on different samples of plants or parts of plants. For example, for uniformity in wheat (see UPOV Test Guidelines for Wheat: TG/3), some characteristics are observed on a sample of 2,000 plants, whilst some other characteristics are observed on a sample of 100 parts of plants taken from 100 plants. Off-type plants observed in the plot of 2,000 plants can be excluded from further observations. For the plant parts taken from 100 plants, it is not normally possible to trace back the plant part to the original plant in the plot. Therefore, the sample of 100 plant parts needs to be considered to be independent from the 2,000 plants. Another independent sample of the variety is observed for seed characteristics. In such cases, a uniformity assessment should be carried out on all the independent samples, using the appropriate population standard. A variety should be considered to be uniform if the uniformity requirements are fulfilled in all samples.

6.4 Off-types and standard deviations

In some cases, the uniformity of a variety may be determined on the basis of off-types for some characteristics and standard deviations for other characteristics. For example, in carrot (see UPOV Test Guidelines for Carrot: TG/49), many root characteristics are observed visually. Those root characteristics are visually observed on the same sample of 200 plants and off-types are determined on the basis of all the visually observed root characteristics. Certain root characteristics can be observed visually or by measurement: root length, root width and root weight. Where measurements are used for those characteristics, the UPOV Test Guidelines recommend that the measurements are based on 60 plants. In this situation,

the standard deviation approach is applied individually for each of the three measured characteristics. The sample of 60 roots will not contain any roots which have been identified as off-types by visual observation. However, because the observations on leaves is made before the observations on the roots, the sample of 60 leaves taken for the measurement of leaf length could contain leaves of plants which are off-type plants on the basis of root characteristics. A variety should be considered to be uniform if the uniformity requirements are fulfilled in all samples.

~~These examples illustrate, that the assessment of uniformity is linked to the individual samples and the order in which the characteristics are observed. If the observations are done on different samples the assessment of uniformity of a variety is more complex and appropriate decision rules have to be developed.~~

Abbreviations: CAJ: Administrative and Legal Committee
TC: Technical Committee
TC-EDC: Enlarged Editorial Committee
TWA: Technical Working Party for Agricultural Crops
TWC: Technical Working Party on Automation and Computer Programs
TWF: Technical Working Party for Fruit Crops
TWO: Technical Working Party for Ornamental Plants and Forest Trees
TWV: Technical Working Party for Vegetables

[a] New text proposed by the TWA.
[b] Proposal made by Doug Waterhouse (Australia) to TC-EDC to delete text.
[c] "[cross ref.]" will be deleted on adoption of the document.
[d] Proposal made by Doug Waterhouse (Australia) to TC-EDC to add text in square brackets.
[e] The TWC proposed to clarify that the environmental variation has two components: the environmental component and the observer/technical component. The modified text was proposed by the TWA.
[f] Document TGP/9/1 Draft 9 states that "2.3.4.2 […] as a general rule, [the states of expression of] qualitative characteristics are not influenced by the environment[f] (see General Introduction, Chapter 4.4.1) […]".
[g] The TWA proposed to consider providing an example for vegetatively propagated varieties. The TWF proposed to add apricot and avocado.
[h] The TC agreed to list [examples of] truly self-pollinated and mainly self-pollinated types separately.
[i] Doug Waterhouse (Australia) commented to the TC-EDC that the phrase was not necessarily correct and should be amended.
[j] The order of the second and third sentences has been reversed as proposed by the TWA.
[k] Addition proposed by the TWA.
[l] Sally Watson (TWC Chairperson) commented to the TC-EDC that "the expression 'range of variation' is used. Even though it has been used in the General Introduction, it is not correct. It should be replaced with 'level of variation'."
[m] Addition proposed by the TWA.
[n] Amendment proposed by Doug Waterhouse (Australia) to TC-EDC to align text with that in TGP/9/1 Draft 9, Section 5.3.
[o] Addition proposed by Doug Waterhouse (Australia) to TC-EDC.
[p] The TC agreed that information on the assessment of uniformity when multiple locations are used and guidance concerning bulk samples should be provided. It is proposed that those matters will be included in TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability".
[q] Chris Barnaby (drafter of Section 4) proposed to the TC-EDC to replace "very little" with "reduced".
[r] The TC-EDC agreed that the section on the use of off-types and standard deviations for the same variety should be covered by a new Section 6.
[s] The TC agreed that a section on the determination of off-types should be included in the draft of TGP/10/1 to be considered by the Technical Working Parties in 2006. The TC has not reviewed the text in Sections 4.2 and 4.3.
[t] The TWO and TWF proposed to restructure the section on the basis of plants which should not be considered as off-types (Section 4.2.3) and plants which should be considered as off-types (Sections 4.2.4 and 4.2.5).
[u] The TWO proposed that the list should be presented in a non-restrictive way. To read "The cause of an observed atypical expression may not be the result of genetic expression but as a result of an external factor. It is important to differentiate between genetic causes of atypical expression and external causes of atypical expression such as environment, damage and cultural practice. Examples of external factors which may cause atypical expression include: …"
[v] Text proposed by Chris Barnaby (drafter of Section 4).
[w] Text modified as proposed by the TWO.
[x] The TWA proposed that the section should explain that it may be necessary to undertake further investigations to determine whether atypical plants were off-types.
[y] The TWF and TWO considered that it was not possible to clearly separate between whole plant off-types (Section 4.2.4) and plant-part off-types (Section 4.2.5): off-types are considered on a characteristic-by-characteristic basis, in the same way as for the assessment of distinctness, as presented in Section 4.2.2. The TWC, TWF and TWO considered that paragraph 4.2.4.2 should be deleted. The TWF considered that paragraph 4.2.4.3 should be moved to the section which should not be considered off-types (the present section 4.2.3). The TWO proposed that, when restructuring the section, special attention should be given to the

situation presented in paragraph 4.2.5.3. The TWO proposed to have the title "Guidance for determining off-types" after merging Sections 4.2.4 and 4.2.5.

[z] Reworded text proposed by the TWO for final sentence.

[aa] With respect to the two versions presented, the TWA, TWF, TWO and TWV proposed to retain version 2 (as presented in TGP/10/1 Draft 6). The TWA supported version 2 on the basis that this would promote a more harmonized approach within UPOV, whilst still allowing some flexibility for exceptional cases.

[bb] Covered by Section 4.5.

[cc] Doug Waterhouse (Australia) proposed to the TC-EDC that shoot color was a poor example and flower color would be better (see Section 4.2.2.1).

[dd] Doug Waterhouse (Australia) proposed to the TC-EDC that paragraphs 4.3.2.4 and 4.3.2.5 should be combined and the text should be reviewed.

[ee] The TWO proposed to explain that the analysis of a further growing cycle or new plant material is related to the uniformity assessment and not to stability. (Note: no change has been made).

[ff] Beate Rücker (Germany) proposed to the TC-EDC that the sentence be deleted.

[gg] The TWF and TWO proposed a subdivision into two paragraphs, one dealing with growing of a further generation and another with the examination of new plant material. (Note: no change has been made because the first sentence covers the existing single paragraph).

[hh] The TWV proposed to explain that measurements might be used to identify off-types where, for example, the observations were done at different times (e.g. time of flowering), but to explain that the use of measurements would reflect off-types which could be observed visually. At the TWA, it was noted that counting was an example of a form of measurement which could be used to identify off-types. It was also noted that it might be possible for "off-types" to be determined by statistical analysis of measurements (e.g. leaf length). However, for such cases, concern was expressed at how the link between the determination of off-types and the standard for distinctness could be achieved. The TWF concluded that there was no need to develop such a section. At the TC-EDC meeting on January 9, 2007, Mr. Niall Green agreed to develop a text for this section.

[ii] Modified text proposed by TWC.

[jj] At the TWA, it was noted that the extract from the General Introduction addressed both mainly self-pollinated varieties and inbred lines of hybrid varieties, which could cause confusion. Therefore, the TWA proposed that further elaboration should be provided to explain that: (i) where appropriate, it was possible for the same tolerance to be used for truly self-pollinated and mainly self-pollinated varieties; and (ii) that an additional tolerance could be accepted for clear cases of out-crossed plants in inbred lines as well as plants obviously resulting from the selfing of a parent line in single-cross hybrids. The TWV proposed to introduce a specific paragraph to explain the higher off-type tolerance for inbred plants in hybrid varieties.

[kk] Addition proposed by the TWA.

[ll] Sally Watson (TWC Chairperson) proposed to the TC-EDC that the text should read "The Test Guidelines recommend for a particular type(s) of variety a general, i.e. "fixed", population standard and an acceptance probability, and provide the maximum acceptable number of off-types for ~~an appropriate~~ a given sample size. The population standard and acceptance probability, together with ~~an appropriate~~ the sample size and maximum acceptable number of off-types, are selected on the basis of experience, in particular with reference to other Test Guidelines for comparable types of variety."

[mm] The TWO agreed that the guide might be extended to cover the number of plants to be examined. That aspect would, for example, cover whether more plants might be appropriate for the examination of varieties which were more likely to contain off-types (e.g. varieties resulting from mutation, variegated varieties, varieties known to contain transposons), in order to allow a suitable assessment of potential off-types. It might also address the selection of the number of plants in relation to the number of off-types allowed in different sample size ranges. (Small sample sizes which do not allow any off-types mean that the occurrence of any chance mutation may cause the rejection of the variety.)

[nn] The TWC proposed to include that care is needed when choosing the sample size in order to produce a good test.

[oo] New text drafted at the request of the TC at its forty-second session (TC/42) when considering TGP/10/1 draft 3. The text was drafted after the TC/42 session and included in TGP/10/1draft 4, which was considered by the Technical Working Parties and the CAJ in 2006. It has not yet been reviewed by the TC.

[pp] Doug Waterhouse (Australia) proposed to the TC-EDC that the term "with comparable expression of characteristics" should be clarified.

[qq] The TWC proposed to remove the reference to long term LSD and to add mention of the 1.26 x standard deviation method in 5.2.1.4 as the alternative name for the 1.6 x variance method. The TWA proposed that the text in brackets read "(1.26 x standard deviations, 1.6 x variance and long-term LSD)".

[rr] Doug Waterhouse (Australia) proposed to the TC-EDC that the text should be modified to avoid suggesting that other techniques were only appropriate where the conditions for COYU were not fulfilled.

[ss] The TWC proposed to remove the reference to long term LSD and to add mention of the 1.26 x standard deviation method in 5.2.1.4 as the alternative name for the 1.6 x variance method. The TWA proposed that the text in brackets read "(1.26 x standard deviations, 1.6 x variance and long-term LSD)".

[tt] Text prepared by Beate Rücker at the request of the TC-EDC (Background: the TWV proposed to explain the cases in cross-pollinated varieties where uniformity is assessed for some characteristics on the basis of off-types and standard deviations, i.e. any off-type plants are identified and then standard deviations are applied (disregarding off-type plants). The TWA heard that there were several crops where varieties were examined using a combination of off-types and standard deviations. It also noted that the table in Section 2.5 indicated that a combination of off-types and standard deviations would probably be needed in cross-pollinated varieties which were examined using quantitative and qualitative and/or pseudo-qualitative characteristics. Therefore, it was agreed that a new Section 6 "Combination of Off-types and Standard Deviations" should be created to provide guidance on the examination of uniformity where a combination of off-types and standard deviations was used. In particular, it was noted that it would be helpful to explain that standards would need to be set for both off-types and standard deviations and that a variety would need to meet both standards. It was also considered important to provide guidance on whether off-type plants should be disregarded from the calculation of standard deviations for some or all characteristics. The TWC also proposed to clarify whether off-types are removed for the calculations for COYU.)

[End of document]