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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/10****EXAMINING UNIFORMITY**

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*and*

*the Council at its fifty-third ordinary session  
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*Disclaimer: this document does not represent UPOV policies or guidance*

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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 distinctness, which must also be considered for uniformity and stability.

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) 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 has both genetic components and environmental components (e.g. temperature, light, soil etc.). 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. As a general rule, the states of expression of qualitative characteristics are not influenced by the environment.

### **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) within vegetatively propagated and truly self-pollinated varieties, a low level of genetic variation is expected. Variation in the expression of characteristics within such varieties should result, predominantly, from environmental influences;

(b) within mainly self-pollinated varieties, a low level of genetic variation caused by some cross pollination is accepted, but variation in the expression of characteristics within such varieties should result, predominantly, from environmental influences. More variation may be tolerated within mainly self-pollinated varieties than for vegetatively propagated and truly self-pollinated varieties;

(c) within cross-pollinated varieties (including synthetic varieties), a higher genetic variation is expected than for vegetatively propagated and self-pollinated varieties. Variation in the expression of characteristics within such varieties results from both genetic and environmental components. The overall level of variation is, therefore, generally higher within cross-pollinated and synthetic varieties than for self-pollinated and vegetatively propagated varieties;

(d) genetic variation within 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 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, 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 level<sup>1</sup> of variation within a variety is greater, 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 level<sup>1</sup> 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 level<sup>1</sup> 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

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<sup>1</sup> The term "level of variation" is considered to be more appropriate than the term "range of variation", which has been used in the General Introduction (see, for example, Chapter 6.4).

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 level<sup>1</sup> of variation, are set by comparison with comparable varieties, or types, already known [...]”.

2.4.2 In addition to the examples provided in the General Introduction for “other than single-cross hybrids” (three-way cross and double cross hybrids), in some cases, a segregation of certain characteristics is acceptable for synthetic varieties and for male sterile varieties maintained by near-isogenic maintainer lines (e.g. segregation of characteristics linked to male sterility), if it is compatible with the method of propagation of the variety.

2.4.3 Thus, for hybrids other than single-cross hybrids, for synthetic varieties and, in some cases, for varieties maintained by near-isogenic maintainer lines, a segregation for certain characteristics, in particular for qualitative characteristics, is acceptable 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 statistical method, such as one based on the  $\chi^2$  test (see document TGP/8).

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

2.4.5 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. Those situations are considered further in Section 6.

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.

| Method of propagation of the variety       | Type of expression of characteristic |                         |   |
|--|--------------------------------------|-------------------------|---|
|  | Qualitative (QL)                     | Pseudo-Qualitative (PQ) | Quantitative (QN)   |
| Vegetatively propagated                    | <i>Off-types</i>                     | <i>Off-types</i>        | <i>Off-types (visual observation)</i><br><i>Standard Deviations (measurement)</i> |
| Self-pollinated                            | <i>Off-types</i>                     | <i>Off-types</i>        | <i>Off-types (visual observation)</i><br><i>Standard Deviations (measurement)</i> |
| Cross-pollinated                           | <i>Off-types</i>                     | <i>Off-types</i>        | <i>Standard Deviations</i>  |
| Single-cross hybrid (in-bred parent lines) | <i>Off-types</i>                     | <i>Off-types</i>        | <i>Off-types (visual observation)</i><br><i>Standard Deviations (measurement)</i> |
| Other hybrids                              | *                                    | *                       | *   |

\* To be considered according to the type of hybrid.

### **SECTION 3: METHOD OF OBSERVATION OF CHARACTERISTICS**

#### **3.1 Off-type approach**

As with the observation of characteristics for distinctness (see document TGP/9 "Examining Distinctness", Section 4.2), 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 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.

#### **3.2 Standard deviations approach**

3.2.1 As with the observation of characteristics for distinctness (see document TGP/9, Section 4.2), 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 data fulfill the conditions for calculation of the mean and standard deviation;

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

## **SECTION 4: UNIFORMITY ASSESSMENT ON THE BASIS OF OFF-TYPES**

### **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 reference in the General Introduction to the possibility of off-types being determined on the basis of measurements.

### **4.2 Determination of Off-types by visual assessment**

#### **4.2.1 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 [General Introduction] 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.

#### **4.2.2 Guidance for determining Off-types**

4.2.2.1 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.

4.2.2.2 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.

4.2.2.3 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.

4.2.2.4 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 in a relevant characteristic 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. However, in some cases, the presence or absence alone of atypical expression for a characteristic may not be sufficient and the frequency and proportion of the atypical expression may also need to be considered (e.g. a single fruit with atypical expression in a relevant characteristic caused by genetic factors in a tree, may not result in an off-type plant).

#### 4.2.3 Investigating plants with atypical expression

4.2.3.1 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 other DUS examiners and the breeder. Consultation with panels of experts, botanists, botanical gardens, plant collectors etc. may also be helpful.

4.2.3.2 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.

4.2.3.3 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. Depending on the circumstances, new plants or plant material may be requested from the breeder and/or plants may be propagated from existing DUS trial material, including from the plants with atypical expression. 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. In cases where a new batch of plants is requested, a sample of the original material should be retained, where possible, to check the conformity of the new material with the original material.

### 4.3 **Determination of Off-types using measurements**

4.3.1 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’.”

4.3.2 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.

### 4.4 **Acceptable number of Off-types**

#### 4.4.1 Self-pollinated, vegetatively propagated and single-cross hybrid varieties

4.4.1.1 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 “acceptance probability” is the minimum probability of accepting as uniform a variety with the population standard of off-types.<sup>2</sup>

4.4.1.2 As explained in Section 2, 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.

4.4.1.3 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.

4.4.1.4 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 acceptable number of off-types for

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<sup>2</sup> This explanation of the term “acceptance probability” is considered to be more appropriate than the explanation for “acceptance probability” which has been used in the General Introduction (see, for example, Chapter 6.4.1.3).

an appropriate sample size. The population standard and acceptance probability, together with an appropriate sample size, are selected on the basis of experience, in particular with reference to other UPOV Test Guidelines for comparable types of variety.

4.4.1.5 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.

4.4.1.6 Larger sample sizes 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 uniformity.

4.4.1.7 Detailed guidance on the use of the off-type approach, including tables of maximum acceptable numbers of off-types for given sample sizes corresponding to fixed population standards and acceptance probabilities, is provided in document TGP/8.

#### 4.4.2 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 level of uniformity found in comparable varieties.

### 4.5 **Setting standards for new types and species**

4.5.1 As explained in Section 4.4.1.5, 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). 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").

4.5.2 Setting the uniformity standard too high could lead to the rejection of a variety which, because of its features of propagation, could not be expected to meet that standard.

### 4.6 **Assessing uniformity by off-types on the basis of more than one growing cycle**

4.6.1 Two independent growing cycles could take place in a single location in different years, or in different locations in the same year, according to document TGP/8 Part I, Sections 1.2 and 1.3.

4.6.2 The following guidance is not intended to be used for the assessment of uniformity by off-types on the same plants in two growing cycles. Results from growing cycles using different lots of plant material should not be combined.

#### 4.6.3 Approach 1: Third growing cycle in the case of inconsistent results

4.6.3.1 A variety is considered uniform if it is within the uniformity standard in both of the two growing cycles.

4.6.3.2 A variety is considered non-uniform if it fails to meet the uniformity standard in both of the two growing cycles.

4.6.3.3 If at the end of the two growing cycles the variety is within the uniformity standard in one growing cycle but is not within the uniformity standard in the other growing cycle, then uniformity is assessed in a third growing cycle. If in the third growing cycle the variety is within the uniformity standard, the variety is considered uniform. If in the third growing cycle the variety fails to meet the uniformity standard, the variety is considered non-uniform.

4.6.3.4 Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle. It is important to identify whether differences in number of off-types between growing cycles were due to environmental reasons or sampling variation.

4.6.3.5 Furthermore, if in the first growing cycle a variety exceeds a predefined upper limit of off-types the variety may be rejected after a single growing cycle.

#### 4.6.4 Approach 2: Combining the results of two growing cycles in the case of inconsistent results

4.6.4.1 A variety is considered uniform if it is within the uniformity standard in both of the two growing cycles.

4.6.4.2 A variety is considered non-uniform if it fails to meet the uniformity standard in both of the two growing cycles.

4.6.4.3 If at the end of the two growing cycles the variety is within the uniformity standard in one growing cycle but is not within the uniformity standard in the other growing cycle, a variety is considered uniform if the total number of off-types at the end of the two growing cycles does not exceed the number of allowed off-types for the sample size of growing cycles 1 and 2 combined.

4.6.4.4 Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle. A statistical test for consistency should be applied when appropriate. It is important to identify whether differences in number of off-types between growing cycles were due to environmental reasons or sampling variation.

4.6.4.5 Furthermore, if in the first growing cycle a variety exceeds a predefined upper limit of off-types the variety may be rejected after a single growing cycle.

#### 4.6.5 Approach 3: Combining the results of two growing cycles

4.6.5.1 A variety is considered uniform if the total number of off-types at the end of the two growing cycles does not exceed the number of allowed off-types for the combined sample.

4.6.5.2 A variety is considered non-uniform if the total number of off-types at the end of the two growing cycles exceeds the number of allowed off-types for the combined sample.

4.6.5.3 A variety may be rejected after a single growing cycle, if the number of off-types exceeds the number of allowed off-types for the combined sample (over two cycles).

4.6.5.4 Care is needed when considering results that are very different in each of the growing cycles, such as when a type of off-type is observed at a high level in one growing cycle and is absent in another growing cycle. A statistical test for consistency should be applied when appropriate. It is important to identify whether differences in number of off-types between growing cycles were due to environmental reasons or sampling variation.

*Example:*

Population Standard = 1%  
Acceptance Probability  $\geq$  95%

|   |
|---|
| Sample Size in each of growing cycles 1 and 2 = 50<br>Maximum number of Off-Types = 2   |
| Sample Size in growing cycles 1 and 2 combined = 100<br>Maximum number of Off-Types = 3 |

|                     | Growing cycle |        | Decision             |              |              |
|---------------------|---------------|--------|----------------------|--------------|--------------|
|                     | First         | Second | Approach 1           | Approach 2   | Approach 3   |
| Number of Off-Types | 1             | 1      | uniform              | uniform      | uniform      |
|                     | 2             | 2      | uniform              | uniform      | non-uniform  |
|                     | 0             | 3*     | third growing cycle* | uniform*     | uniform*     |
|                     | 1             | 3*     | third growing cycle* | non-uniform* | non-uniform* |
|                     | 1             | 4*     | third growing cycle* | non-uniform* | non-uniform* |
|                     | 4**           | 1*     | third growing cycle* | non-uniform* | non-uniform* |

\* Care is needed when considering results that were very different in each of the growing cycles, such as when a type of off-type was observed at a high level in one growing cycle and was absent in another growing cycle. A statistical test for consistency should be applied when appropriate. It is important to identify whether differences in number of off types between growing cycles were due to environmental reasons or sampling variation.

\*\* If in the first growing cycle a variety exceeds a predefined upper limit of off-types the variety may be rejected after a single growing cycle.

#### **4.7 Assessing uniformity by off-types on the basis of sub-samples within a single test/trial**

##### **4.7.1 Approach: Use of sub-sample as a first step of assessment**

4.7.1.1 A variety is considered uniform if the number of off-types does not exceed a predefined lower limit in the sub-sample.

4.7.1.2 A variety is considered non-uniform if the number of off-types exceeds a predefined upper limit in the sub-sample.

4.7.1.3 If the number of off-types is between the predefined lower and upper limits, the whole sample is assessed. The lower and upper limits have to be chosen considering comparable type I and type II errors in the sub-sample and the whole sample.

*Example:*

In a sample size of 100 plants, the acceptable number of off-types is 3 (based on a population standard of 1% and an acceptance probability of at least 95%).

In a subsample of 20 plants used in the context of the sample size of 100 plants above:

- A variety is considered uniform if no off-types are observed in the sub-sample.
- A variety is considered non-uniform if the number of off-types in the sub-sample exceeds 3.
- If the number of off-types is 1 to 3, the whole sample of 100 plants is assessed.
- If the number of off-types in the sample of 100 plants exceeds 3, the variety is considered non-uniform.

## 4.8 Plants which are not considered as Off-types

### 4.8.1 Atypical plants which are not considered to be Off-types

4.8.1.1 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 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) physical damage (e.g. due to environmental conditions (sun, wind, precipitation, frost), chemical application (e.g. herbicide scorch) etc. ;
- (e) lack of pollination; (e.g. in strawberry, poor and uneven pollination can result in misshapen fruit);
- (f) graft incompatibility (e.g. Graft incompatibility in *Gymnocalycium mihanovichii* (Chin Cactus) can change the color of the scion); and
- (g) propagation effects.

4.8.1.2 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.

### 4.8.2 Within-plant variation which does not indicate an Off-type plant

4.8.2.1 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 can be genetically based. 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.

4.8.2.2 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.

### 4.8.3 Further investigation

Determining whether an atypical plant or within-plant variation should be considered to constitute an off-type plant may require further investigation (see Section 4.2.3).

## **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 high level<sup>1</sup> 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 level<sup>1</sup> 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 level<sup>1</sup> 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.

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 for each characteristic on the basis of varieties within the same trial with comparable expression for that characteristic.

5.2.3 Details of the COYU method are provided in document TGP/8.

5.2.4 Information on other appropriate statistical methods is provided in document TGP/8.

### **5.3 Setting standards for new types and species**

As explained in Section 5.1, in cases where the off-type approach is not appropriate, relative tolerance limits for the level<sup>1</sup> 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 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). 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).

## **SECTION 6: COMBINING ALL OBSERVATIONS ON A VARIETY**

### **6.1 Introduction**

The uniformity of a variety is assessed by the observation of individual plants for all relevant characteristics. In some crops, all of those characteristics are observed on all plants in the test. In other crops, some of those 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 relevant characteristics and on the basis of standard deviations for other relevant characteristics. Therefore, specific guidance 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.

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