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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

to be considered by the

Technical Working Party for Vegetables (TWV), at its fortieth session, to be held in Guanajuato, Guanajuato State, Mexico, June 12 to 16, 2006

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Administrative and Legal Committee (CAJ), at its fifty-fourth session, to be held in Geneva, Switzerland, on October 16 and 17, 2006

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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 (Chapter 6: Section 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".

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" *[cross ref.]* 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 observation of variation in the expression of relevant characteristics within varieties is the basis for the assessment of uniformity. This variation has both genetic and environmental components. 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.

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 and truly self-pollinated (e.g. rice, soybean, wheat)[1] 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)[1] 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. In relation to self-pollinated, vegetatively propagated and mainly self-pollinated varieties a higher genetic variation is accepted. The overall level of variation is, therefore, generally higher in cross-pollinated and synthetic varieties;

(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 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 (see Chapter 6:

[1] The TC agreed to list [examples of] truly self-pollinated and mainly self-pollinated types separately.

Section 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 of variation within a variety is larger, because of the features of its propagation, and in particular for cross-pollinated, including synthetic, varieties, the plants are not all very similar and it is not possible to visualize which plants should be considered as atypical or "off-types." In this case the uniformity can be assessed by considering the overall range of variation, observed across all the individual plants, to determine whether 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 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: Section 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 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.

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 approach for the assessment of uniformity, although there may be exceptions:

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

* to be considered according to the type of hybrid

SECTION 3: METHOD OF OBSERVATION OF CHARACTERISTICS[2]

3.1 Off-type approach

As with the observation of characteristics for distinctness (see document TGP/9 Section 4.1.4 *[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 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 4.2 *[cross ref.]*.

3.2 Standard deviations approach

3.2.1 As with the observation of characteristics for distinctness (see document TGP/9 Section 4.1.4 *[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 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

As explained in Section 2, the level of variation within varieties depends on both the features of propagation of the variety and the type of expression of the characteristic. Thus, the uniformity of a variety may be determined exclusively by off-types, exclusively by standard deviations, or by off-types for some characteristics and by standard deviations for other characteristics.

[2] 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".

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 possibility of off-types being determined on the basis of measurements.

4.2 Determination of Off-Types by Visual Assessment[3]

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 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 Application of the standard for distinctness

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. That requirement means that an off-type plant could potentially become a distinct variety if it could be propagated unchanged.

4.2.2.2 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. The following guidance 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

[3] 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 have not reviewed the text in Section 4.2.

good level of experience within the genus or species concerned, or within a similar genus or species.

4.2.3 Causes of atypical[4] expression

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. 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;

(c) infection by disease;

(d) pest infestation;

(e) graft incompatibility (example: Graft incompatibility in *Gymnocalycium mihanovichii* (Chin Cactus) can change the color of the scion)

4.2.4 Atypical expression throughout the whole plant

4.2.4.1 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.

4.2.4.2 An off-type plant may be clearly distinguishable for a single characteristic or may be clearly distinguishable for more than one characteristic on a characteristic-by-characteristic basis. However, there can be cases where the expression for individual plant characteristics do not make the plant clearly distinguishable, but, when put together, the differences indicate a plant that is atypical. The small changes present on each individual characteristic combine to form a more clearly observable difference. An example can be seen in apple coloration and patterning. The fruit color, color intensity, amount of overcolor and pattern of overcolor observed individually may not indicate that the apple is clearly distinguishable, however, when the whole apple is observed, the overall impression may be atypical. The definition of an off-type implies that any atypical expression of a characteristic, even if that characteristic is not present in the Test Guidelines, could make a plant an off-type. However, the definition clarifies that any off-type plant must be “clearly distinguishable”.

4.2.4.3 The General Introduction TG/1/3, Section 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

[4] The term “atypical” is synonymous with “non true-to-type”

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 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.

4.2.5 Atypical expression in part(s) of the plant

4.2.5.1 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 not be true-to-type: 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. *[Version 1: When making this decision, the examiner should consider whether or not the plant is clearly distinguishable from the other plants and may take into account the proportion of the plant which is affected. In addition, it would be appropriate to consider whether the part of the plant affected was a part of the plant which would normally be used for propagation of the variety and, therefore, whether it would produce an atypical plant(s) in the next generation.] / [Version 2: 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.]*. 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.

4.2.5.2 An off-type plant could be indicated by the nature, type and frequency of the variation in expression. Thus, in some case, 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 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. All plants of the variety in the trial must be able to be described in the same way according to the Test Guidelines. If this is not possible then the plants in trial do not form a uniform variety.

4.2.5.3 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. 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.

4.2.5.4 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 (e.g. resulted from a mutation).

4.2.6 Investigating plants with atypical expression

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

4.2.6.2 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.

4.2.6.3 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. 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.

4.3 Determination of Off-Types Using Measurements

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"."

Section to be developed?

4.4 Acceptable number of off-types

4.4.1 Self-Pollinated and Vegetatively Propagated Varieties

4.4.1.1 The General Introduction (Chapter 6: Section 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 percentage of off-types to be accepted if all individuals of the variety could be examined. The probability of correctly accepting that a variety is uniform is called the "acceptance probability".

4.4.1.2 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: Section 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 […]."

4.4.1.3 The Test Guidelines recommend for a particular type(s) of variety a general, i.e. "fixed", population standard and acceptance probability and provide the acceptable number of off-types for a given sample size. The population standard and acceptance probability are fixed on the basis of experience, in particular with reference to other Test Guidelines for comparable types of variety.

4.4.1.4 In the absence of Test Guidelines, an appropriate population standard and acceptance probability with the acceptable number of off-types for a given sample size are fixed on the basis of experience, in particular with reference to Test Guidelines for comparable types of variety.

4.4.1.5 Detailed guidance on the use of off-type approach, including tables of maximum numbers of off-types for given sample sizes corresponding to fixed population standards and acceptance probabilities, is provided in document TGP/8 Section 3 *[cross ref.]*.

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 number of off-types found in comparable varieties.

4.5 Setting standards for new types and species

As explained in Section 4.3.1.4 *[cross ref.]*, in the absence of Test Guidelines, an appropriate population standard and acceptance probability with the acceptable number of off-types for a given sample size are fixed on the basis of experience, in particular with reference to 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.

SECTION 5: UNIFORMITY ASSESSMENT ON THE BASIS OF STANDARD DEVIATIONS

5.1 Introduction

The General Introduction (see Chapter 6: Section 6.4) explains that, in cases where there is a wide range 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 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 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

5.2.1.1 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.

5.2.1.2 UPOV has proposed several statistical methods for dealing with uniformity in measured quantitative characteristics. One method, which takes into account variations 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.

5.2.1.3 Details of the COYU method are provided in document TGP/8 Section 2.2 *[cross ref.]*.

5.2.1.4 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. 1.6 × variance, long term LSD). Information on other appropriate statistical methods 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 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.

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