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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/12

**“SPECIAL [GUIDANCE ON CERTAIN] PHYSIOLOGICAL<sup>a</sup> CHARACTERISTICS”**

*prepared by the Office of the Union*

*to be considered by the*

*Technical Committee at its forty-fifth session,  
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Note for Draft version

**Strikethrough (highlighted)** indicates deletion from the text agreed by the Technical Committee (TC) at its forty-fourth session

**Underlining (highlighted)** indicates insertion to the text agreed by the TC at its forty-fourth session

**highlighted** text indicates text which has been developed at the request of the TC at its forty-fourth session, but which has not been considered by the TC

**Footnotes** will be retained in published document

**Endnotes** are for background information when considering this draft and will not appear in the final, published document

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## SECTION I. DEVELOPMENT OF CHARACTERISTICS BASED ON A RESPONSE TO AN EXTERNAL FACTOR

### 1. Introduction

#### 1.1 Requirements for characteristics based on a response to an external factor

1.1.1 The General Introduction (document TG/1/3, Chapter 2, Section 2.5.3) states that:

“The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc. In some cases (e.g. disease resistance), reaction to certain factors is intentionally used (see TG/1/3 Chapter 4, Section 4.6.1) as a characteristic in the DUS examination. However, where the factor is not intended for DUS examination, it is important that its influence does not distort the DUS examination. Accordingly, depending on the circumstances, the testing authority should ensure either that:

- (a) the varieties under test are all free of such factors or,
- (b) that all varieties included in the DUS test, including varieties of common knowledge, are subject to the same factor and that it has an equal effect on all varieties or,
- (c) in cases where a satisfactory examination could still be undertaken, the affected characteristics are excluded from the DUS examination unless the true expression of the characteristic of the plant genotype can be determined, notwithstanding the presence of the factor.”

1.1.2 The General Introduction (document TG/1/3, Chapter 4, Section 4.6.1) further states that “Characteristics based on the response to external factors, such as living organisms (e.g. disease resistance characteristics) or chemicals (e.g. herbicide resistance characteristics), may be used provided that they fulfil the criteria specified in [document TG/1/3, Chapter 4] Section 4.2. In addition, because of the potential for variation in such factors, it is important for those characteristics to be well defined and an appropriate method established which will ensure consistency in the examination.” It should also be noted that, notwithstanding the fact that varieties may exhibit such traits, special tests for characteristics based on response to external factors do not need to be used where the routine characteristics resolve distinctness.

1.1.3 In the case of external factors which are living organisms (~~L.O's~~<sup>b</sup>), certain specific conditions must be considered because of the possible variation of the living organism-L.O.<sup>b</sup> which interacts with the variety. In comparison with climatic or soil factors, additional sources of variation can change the effect of the living organism-L.O.<sup>b</sup> on the variety:

- the effect of factors, such as temperature, relative humidity and light, on the development or the aggressivity of the living organism-L.O.<sup>b</sup>
- the genetic variability of the living organism-L.O.<sup>b</sup> (different pathotypes<sup>1</sup>).

Due to these sources of variation, the protocols used to obtain the description of a candidate variety, or to compare close varieties, must be established with due attention to these sources of variation.

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<sup>1</sup> the term “pathotype” is used in a general way in this document and covers terms such as “race”, “strain” etc., although the terms “race”, “strain” etc. will be used in Test Guidelines where appropriate.

1.1.4 Table 1 presents the basic requirements that a characteristic should fulfill before it is used for DUS testing or producing a variety description together with some particular considerations with regard to characteristics based on the response to external factors.

1.1.5 ~~Chapters~~ Section I, 2 to 4 provides guidance on the use of characteristics based on the response to external factors in the form of disease resistance, insect resistance and chemical response. Characteristics based on the response to other types of external factors may also be appropriate where they take into account the considerations presented in Table 1.

Table 1

| <b>Basic requirements that a characteristic should fulfill<br/>(document TG/1/3 Chapter 4, Section 4.6.1)</b>   | <b>Particular considerations with regard to characteristics based on response to external factors</b>   |
|---|---|
| <i>The basic requirements that a characteristic should fulfill before it is used for DUS testing or producing a variety description are that its expression:</i>  |   |
| <i>(a) results from a given genotype or combination of genotypes;</i>   | knowledge of the nature of genetic control of the response is important   |
| <i>(b) is sufficiently consistent and repeatable in a particular environment;</i>   | <p>(i) important to standardize, as far as possible, the conditions in the field, greenhouse or laboratory, as appropriate, and the methodology used;</p> <p>(ii) the methodology should be validated, e.g. by a ring test; and</p> <p>(iii) the key requirements should be set out in a protocol.</p>  |
| <i>(c) exhibits sufficient variation between varieties to be able to establish distinctness;</i>  | the response and suitable states of expression should be described (see (d) below)  |
| <i>(d) is capable of precise definition and recognition;</i>  | <p>(i) the external factor should be clearly defined and characterized (e.g. disease inoculum, fungal pathotype<sup>2</sup>, virus pathotype, insect biotype, chemical etc.);</p> <p>(ii) the type of response to the external factor (e.g. disease: susceptible / intermediate resistant / resistant; abiotic factors: sensitive / tolerant, etc.) and suitable states of expression (e.g. resistant or susceptible (qualitative characteristic); or levels of resistance / susceptibility (quantitative or pseudo-qualitative characteristic)) should be clearly defined.</p> <p><del>In general, for DUS purposes, “tolerance” is not a suitable characteristic in relation to disease resistance.</del><sup>c</sup></p> |
| <i>(e) allows uniformity requirements to be fulfilled;</i>  | the uniformity requirements for characteristics based on the response to external factors are the same as for other characteristics. In particular, it is necessary for the method to allow the examination of individual plants.   |
| <i>(f) allows stability requirements to be fulfilled, meaning that it produces consistent and repeatable results after repeated propagation or, where appropriate, at the end of each cycle of propagation.</i> | the stability requirements for characteristics based on the response to external factors are the same as for other characteristics.   |

<sup>2</sup> the term “pathotype” is used in a general way in this document and covers terms such as “race”, “strain” etc., although the terms “race”, “strain” etc. will be used in Test Guidelines where appropriate.

## 1.2 Terms Describing the Response of Plants to Pests, Pathogens ~~or~~ and Abiotic Stresses Terminology in Disease Resistance (Definition of the Terms Describing the Reaction of Plants to Pests or Pathogens and to Abiotic Stresses) <sup>d</sup>

### 1.2.1 Preamble

Differing degrees of specificity exist in the relations between plants and pests or pathogens. Identification of such specificity generally requires the use of highly elaborate analytical methods. Recognizing whether a plant is subject to a pest or pathogen or not may depend on the analytical method employed. It is important, in general, to stress that the specificity of pests or pathogens may vary over time and space, depends on environmental factors, and that new pest biotypes or new types of pathogen (pathotypes) <sup>d</sup> capable of overcoming resistance may emerge.

### 1.2.2 Definitions

The following definitions are intended for the purpose of the examination of DUS:

#### 1.2.2.1 Biotic factors (pest or pathogen)

*Immunity:* not subject to infection by a specified pest or pathogen.

*Resistance:* the ability of a plant variety to restrict the growth and development of a specified pest or pathogen and/or the damage they cause when compared to susceptible plant varieties under similar environmental conditions and pest or pathogen pressure. Resistant varieties may exhibit some disease symptoms or damage under heavy pest or pathogen pressure.

*Susceptibility:* is the inability of a plant variety to restrict the growth and development of a specified pest or pathogen.

<sup>e</sup>*Tolerance:* is the ability of a plant to limit the negative effects of a specified pest or pathogen. Effects should be related to aspects such as yield reduction.

In many instances, for DUS purposes, tolerance may not be a suitable characteristic because the method required to establish different levels of tolerance (~~i.e. yield reduction~~) requires a method of examination beyond the normal scope of a DUS test in one place in a limited number of replicates.

#### 1.2.2.2 Abiotic factors (e.g. chemical, temperature)

*Tolerance:* the ability of a plant variety to endure abiotic stress, without serious consequences for growth, appearance and yield.

*Sensitivity:* the inability of a plant variety to endure abiotic stress without serious consequences for growth, appearance and yield.

~~f 1.3 Possible use of gene-specific molecular markers as predictors of traditional characteristics~~

~~UPOV has considered the possibility of using gene-specific molecular markers as a predictor of traditional characteristics in order to avoid the need for examination in a growing trial of characteristics which may be difficult and/or expensive to observe in a growing trial. The situation in UPOV concerning the use of such an approach, known as an “Option 1(a)” approach, is set out in documents TC/38/14 CAJ/45/5 and TC/38/14 Add. CAJ/45/5 Add.. Those documents clarify that a number of assumptions would need to be checked before the use of such an approach, including the need to establish that there was a reliable linkage between any gene specific marker and the expression of the disease resistance characteristic concerned.~~

## 2. Disease Resistance

### 2.1 Introduction

Resistance to pests and diseases ~~in an important breeding aim. Where there is particular focus on breeding for such resistances, the use of disease resistance characteristics in the examination of DUS may be important. However, such characteristics~~<sup>d</sup> poses particular challenges, in particular with regard to the precise definition and recognition of characteristics and ensuring sufficient consistency and repeatability. The following sections address those requirements and the other requirements that a characteristic is required to fulfill.

### 2.2 Criteria for use of disease resistance characteristics

In general, the requirements set out in Table 1 can be fulfilled but a number of requirements pose specific problems:

#### 2.2.1 *Results from a given genotype or combination of genotypes (see Table 1 (a))*

Knowing which genes are responsible for resistance and if it concerns a single gene or a combination of genes gives valuable information that will help to properly observe and evaluate the resistance. Cooperation with breeders also results in better knowledge on the genetic background of the various forms of disease resistance.

#### 2.2.2 *Is sufficiently consistent and repeatable in a particular environment (see Table 1 (b))*

Repeated tests and ring tests have shown that, subject to the use of an appropriate protocol (see [Section I, 2.2.4.4 \[cross ref.\]](#)), the consistency and repeatability of disease resistance for a particular pathotype can be very good.

#### 2.2.3 *Exhibits sufficient variation between varieties to be able to establish distinctness (see Table 1 (c))*

Disease resistance characteristics, if properly tested, can give a clear differentiation in the variety collections. The differentiation may take place at the pathotype level because many collections of varieties are known to show different resistance reactions to different pathotypes of the disease. Guidance on the development of disease resistance as a qualitative or quantitative characteristic is provided in [Section I, 2.3 \[cross ref.\]](#).

#### 2.2.4 *Is capable of precise definition and recognition (see Table 1 (d))*

2.2.4.1 The definition of the disease itself usually does not create problems, for the proper denomination internationally accepted standards may be used such as that of the American Phytopathological Society (APS), for fungi and bacteria, and the International Committee for Taxonomy of Viruses (ICTV) for viruses.

2.2.4.2 The same pathotype may be named differently in different parts of the world, e.g. *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomato, where race 1 in the United States of America is identical to race 0 in Europe. Also, different pathotypes may have the same name, e.g. *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomato, where race 2 in the USA is different from race 2 in Europe. At the moment a joint effort is made by International Seed Federation (ISF) on this subject with the aim to create one clear system of definition and



nomination. The core of this system is the precise definition of a set of host differential lines/varieties with which the pathotypes can be determined. The seed industry is often willing to cooperate by maintaining the necessary stocks of seed for this purpose.

~~§ 2.2.4.3 In Section 1.2, [cross ref.] the definition of the various terms as developed and used by ISF is given. Those definitions can also be found on the ISF website (see [http://www.worldseed.org/cms/medias/file/TradeIssues/PhytosanitaryMatters/PathogenCoding/RecommendedCodesForPestOrganisms/Recommended\\_Codes\\_for\\_Pest\\_Organisms\\_20080301\\_\(En\).pdf](http://www.worldseed.org/cms/medias/file/TradeIssues/PhytosanitaryMatters/PathogenCoding/RecommendedCodesForPestOrganisms/Recommended_Codes_for_Pest_Organisms_20080301_(En).pdf)).<sup>h</sup>~~

2.2.4.34 Ring tests have shown that a set of standards needs to be included in the trial, in order for the observations and evaluation of the results to be harmonized. However, slight differences in the standards, due to differences between lots, can cause problems. To avoid such problems, it is recommended to develop a centralized set of standards for each disease or pathotype. The seed industry is often willing to cooperate by maintaining the necessary stocks of seed for this purpose.

2.2.5 *Allows uniformity requirements to be fulfilled (see Table 1 (e))*

The development of plants is influenced by the environment and the quality of the inoculum. The inoculation, and the interaction between the symptoms and the development of the plant, may cause variation within the trial. Such variation should not be assumed to be the result of a lack of uniformity of the variety (see document TGP/10/1, Section 4.6 [cross ref.] )

2.2.6 *Additional points for consideration*

As additional points for consideration, the following has to be taken into account:

- (i) the availability of reliable inoculum and host differential set

In general, a few institutes maintain stocks of inoculum of most of the diseases that are used in breeding programs. In the explanation of the methods in the Test Guidelines, the available information on these sources should be indicated. If inoculum from another source is used, a defined host differential set should be used to clearly identify the inoculum.

- (ii) quarantine regulations

Some diseases, for which resistance is used for DUS testing by some members of the Union, might be considered as quarantine diseases in other territories. This often means that the import of inoculum and, therefore the disease resistance test, is not possible in certain territories. In such cases, it is possible to use cooperation in DUS examination to overcome the problem (see the “Introduction” to document TGP/5 “Experience and Cooperation in DUS Testing”).

- (iii) technical requirements

The technical requirements of disease tests can, for some DUS testing authorities, be an obstacle for the use of such characteristics. In such cases, ~~it is possible to use~~ cooperation in DUS examination is a means to overcome the problem (see the “Introduction” to document TGP/5 “Experience and Cooperation in DUS Testing”).

## 2.3 Developing characteristics for disease resistance

In general, disease resistance characteristics are qualitative or quantitative characteristics:

### 2.3.1 *Qualitative characteristics*

Disease resistances which are discontinuously expressed as absent or present are qualitative characteristics.

Example: Resistance to downy mildew (*Bremia lactucae*) in Lettuce  
(UPOV Test Guidelines: TG/13/10)

|             | English                           | français                     | Deutsch                                 | español                      | Example Varieties | Note |
|-------------|-----------------------------------|------------------------------|---|------------------------------|-------------------|------|
| <b>39.</b>  | <b>Resistance to downy mildew</b> | <b>Résistance au mildiou</b> | <b>Resistenz gegen Falschen Mehltau</b> | <b>Resistencia al mildiú</b> |                   |      |
| (+)         | ( <i>Bremia lactucae</i> )        | ( <i>Bremia lactucae</i> )   | ( <i>Bremia lactucae</i> )              | ( <i>Bremia lactucae</i> )   |                   |      |
| <b>39.1</b> | <b>Isolate Bl 2</b>               | <b>Isolat Bl 2</b>           | <b>Isolat Bl 2</b>                      | <b>Aislado Bl 2</b>          |                   |      |
| <b>QL</b>   | absent                            | absente                      | fehlend                                 | ausente                      | [...]             | 1    |
|             | present                           | présente                     | vorhanden                               | presente                     | [...]             | 9    |

### 2.3.2 *Quantitative characteristics*

2.3.2.1 Disease resistances for which there is a continuous range of levels of susceptibility / resistance across varieties, are quantitative characteristics. In general, it is not possible to define nine states of resistance which would be necessary in order to apply the standard “1-9” scale. Therefore, the condensed “1-3” scale may be the most appropriate way in which to present such characteristics.

Example: Resistance to *Sphaerotheca fuliginea* (*Podosphaera xanthii*)  
(Powdery mildew) in Melon (UPOV Test Guidelines: TG/104/5)

|             | English  | français  | Deutsch  | español  | Example Varieties | Note |
|-------------|--|---|--|--|-------------------|------|
| <b>70.</b>  | <b>Resistance to <i>Sphaerotheca fuliginea</i></b> | <b>Résistance à <i>Sphaerotheca fuliginea</i></b> | <b>Resistenz gegen <i>Sphaerotheca fuliginea</i></b> | <b>Resistencia a <i>Sphaerotheca fuliginea</i></b> |                   |      |
| (+)         | ( <i>Podosphaera xanthii</i> )<br>(Powdery mildew) | ( <i>Podosphaera xanthii</i> )<br>(Oïdium)        | ( <i>Podosphaera xanthii</i> )<br>(Echter Mehltau)   | ( <i>Podosphaera xanthii</i> )<br>(Oidio)          |                   |      |
| <b>70.1</b> | <b>Race 1</b>                                      | <b>Pathotype 1</b>                                | <b>Pathotyp 1</b>                                    | <b>Raza 1</b>                                      |                   |      |
| <b>QN</b>   | susceptible  | sensible  | anfällig   | susceptible  | [...]             | 1    |
|             | moderately resistant                               | moyennement résistant                             | mäßig resistent                                      | moderadamente resistente                           | [...]             | 2    |
|             | highly resistant                                   | hautement résistant                               | hochresistent  | altamente resistente                               | [...]             | 3    |

2.3.2.2 <sup>j</sup> The “1-3” scale recognizes that, ~~for vegetatively propagated and self-pollinated varieties (see document TGP/9, Sections 5.2.3.2.3 5.2.3.9 to 15 [cross ref.]~~, a difference of two Notes is an appropriate basis for distinctness if the comparison between two varieties is performed at the level of Notes obtained from the growing trial (see document TGP/9, Section 5.2.3.2.3 [cross ref.]). If the difference is only one Note, both varieties could be very close to the same border line (e.g. high end of Note 2 and low end of Note 3) and the difference might not be clear. Thus, only pairs of varieties which are susceptible (Note 1) and highly resistant (Note 3) should be considered distinct on the basis of Notes.

<sup>k</sup> 2.3.2.3 In some cross-pollinated ~~agricultural~~ species (e.g. Lucerne) disease resistance (e.g. resistance to *Colletotrichum trifolii*) is ~~often~~ assessed as ~~the~~ percentage of resistant plants within the population. In those cases a continuous range of variation could be observed ~~in the levels of susceptibility/resistance~~ across varieties. This can be treated as a true quantitative characteristic (1-9 scale) and appropriate statistical methods can be applied in the analysis of data.

Example: Resistance to *Colletotrichum trifolii* in Lucerne  
(UPOV Test Guidelines: TG/6/5)

|                   | English   | français   | Deutsch   | español  | Example Varieties | Note |
|-------------------|---|--|---|--|-------------------|------|
| <b>19. VS (+)</b> | <b>Resistance to <i>Colletotrichum trifolii</i></b> | <b>Résistance à <i>Colletotrichum trifolii</i></b> | <b>Resistenz gegen <i>Colletotrichum trifolii</i></b> | <b>Resistencia al <i>Colletotrichum trifolii</i></b> |                   |      |
| <b>QN</b>         | very low  | très faible  | sehr gering   | muy baja   | [...]             | 1    |
|                   | low   | faible   | gering  | baja   | [...]             | 3    |
|                   | medium  | moyenne  | mittel  | media  | [...]             | 5    |
|                   | high  | élevée   | hoch  | alta   | [...]             | 7    |
|                   | very high   | très élevée  | sehr hoch   | muy alta   | [...]             | 9    |

## 2.4 Explanations for disease resistance characteristics in Test Guidelines

2.4.1 Where disease resistance characteristics are included in Test Guidelines, the following information should be provided in Chapter 8 “Explanations on the Table of Characteristics”:

- (a) nature of the genetic control of disease resistance;
- (b) information on the disease pathotypes;
- (c) source(s) of disease inoculum;
- (d) the host differential set of varieties / lines to use to check the inoculum on correctness regarding the pathotypes used;
- (e) source(s) of host differential set of varieties / lines;
- (f) method for maintaining the disease inoculum;
- (g) test method;
- (h) scoring procedure for determination of states of expression (notes);
- (i) example varieties (pathotype-specific standard varieties); and
- (j) source(s) of example varieties (pathotype-specific standard varieties).

2.4.2 For further guidance, the explanations for the disease resistance characteristics provided as examples in this section can be found in the relevant Test Guidelines.

### 3. Insect Resistance

#### 3.1 Developing characteristics for insect resistance

~~In general, insect resistance characteristics are qualitative or quantitative characteristics. The following examples of insect resistance characteristics are provided for illustrative purposes.~~<sup>d</sup>

#### 3.2 Example of Corn borer (*Ostrinia nubilalis* (Hübner)) resistance in maize varieties

The following example concerns corn borer resistance (*Ostrinia nubilalis* (Hübner)) in maize varieties. The procedure involves a bioassay approach based on the death rate of larvae.

|  | English   | français   | Deutsch   | español  | Example Varieties | Note |
|--|---|--|---|--|-------------------|------|
|  | <b>Resistance to <i>Ostrinia Nubilalis</i> Hübner</b> | <b>Résistance à <i>Ostrinia Nubilalis</i> Hübner</b> | <b>Resistenz gegen <i>Ostrinia Nubilalis</i> Hübner</b> | <b>Resistencia al <i>Ostrinia Nubilalis</i> Hübner</b> |                   |      |
| <b>QN</b><br><sup>d</sup><br><b>QL</b> | susceptible<br><u>absent</u>                          | sensible<br><u>absente</u>                           | anfällig<br><u>fehlend</u>                              | susceptible<br><u>ausente</u>                          | [...]             | 1    |
|  | present   | présente   | vorhanden   | presente   | [...]             | 9    |

#### 3.3 Example of resistance to *Therioaphis maculata* in Lucerne (UPOV Test Guidelines: TG/6/5)

<sup>l</sup> In some cross-pollinated agricultural species (e.g. Lucerne) insect resistance (e.g. *Therioaphis maculata*) is often assessed as the percentage of resistant plants within the population. In those case a continuous range of variation could be observed in the levels of susceptibility/resistance across varieties. This can be treated as a true quantitative characteristic (1-9 scale) and appropriate statistical methods can be applied in the analysis of data.

|                     | English  | français  | Deutsch  | español   | Example Varieties | Note |
|---------------------|--|---|--|---|-------------------|------|
| <b>22. VS C (+)</b> | <b>Resistance to <i>Therioaphis maculata</i></b> | <b>Résistance à <i>Therioaphis maculata</i></b> | <b>Resistenz gegen <i>Therioaphis maculata</i></b> | <b>Resistencia al <i>Therioaphis maculata</i></b> |                   |      |
| <b>QN</b>           | very low   | très faible                                     | sehr gering  | muy baja  | [...]             | 1    |
|                     | low  | faible  | gering   | baja  | [...]             | 3    |
|                     | medium   | moyenne   | mittel   | media   | [...]             | 5    |
|                     | high   | élevée  | hoch   | alta  | [...]             | 7    |
|                     | very high  | très élevée                                     | sehr hoch  | muy alta  | [...]             | 9    |

3.4 Example of resistance to colonization by *Aphis gossypii* in Melon (UPOV Test Guidelines: TG/104/5)

|               | English  | français  | Deutsch   | español  | Example Varieties | Note |
|---------------|--|---|---|--|-------------------|------|
| <b>72. VG</b> | <b>Resistance to colonization by <i>Aphis gossypii</i></b> | <b>Résistance à la colonisation par <i>Aphis gossypii</i></b> | <b>Resistenz gegen Befall durch <i>Aphis gossypii</i></b> | <b>Resistencia a la colonización por <i>Aphis gossypii</i></b> |                   |      |
| (+)           |  |   |   |  |                   |      |
| <b>QL</b>     | absent   | absente   | fehlend   | ausente  | [...]             | 1    |
|               | present  | présente  | vorhanden   | presente   | [...]             | 9    |

3.5 Explanations for insect resistance characteristics in Test Guidelines

3.5.1 Where insect resistance characteristics are included in Test Guidelines, the following information should be provided in Chapter 8 “Explanations on the Table of Characteristics”:

- (a) nature of the genetic control of insect resistance;
- (b) information on the biotypes;
- (c) source(s) of colonies;
- (d) method for maintaining the colonies;
- (e) test method;
- (f) scoring procedure for determination of states of expression (notes); and
- (g) example varieties.

3.5.2 For further guidance, the explanations for the insect resistance characteristics provided as examples in this section can be found in the relevant Test Guidelines.

## 4. Chemical Response

### 4.1 Introduction

Plant growth can be significantly influenced by a number of chemical compounds. When applied to plants, such chemicals can affect the phenology, physiology and change phenotypic characteristics. They include herbicides, plant growth regulators, defoliant, rooting compounds, and compounds used in tissue culture media. Some examples of the effect of herbicides and plant growth regulators on plants and the use of those responses as characteristics in the DUS examination are discussed in this Section.

### 4.2 Herbicides

#### 4.2.1 *Herbicide Tolerant Varieties*

**4.2.1.1** The breeding of herbicide tolerant varieties is now commonplace. When such varieties are treated with herbicide, their level of “tolerance” is manifested by some phenotypic expression(s). Subject to the fulfillment of the requirements for a characteristic to be used in DUS testing (TG/1/3 Section 4.2) these characteristics can be useful in assessing distinctness.

~~<sup>m</sup> 4.2.1.2 Herbicide tolerance can either be an inherent characteristic of a plant variety or can be introduced by conventional plant breeding, mutation, or genetic modification. For example, some grasses are inherently tolerant to 2,4 D (2,4-dichlorophenoxyacetic acid) and other growth hormone mimics. Selection within these grass species has resulted in tolerant varieties. In contrast, other crops may not possess natural tolerance, even at very low levels and genetic modification is required to introduce herbicide tolerance (eg to phosphinothricin or glyphosate).~~

#### <sup>n</sup> 4.2.2 *Case Study on the Use of Herbicide Tolerance in Cotton as a Characteristic in the DUS Examination*

4.2.2.1 Herbicide tolerance which is discontinuously expressed as absent or present is a qualitative characteristic. In glyphosate tolerant genetically modified cotton varieties, tolerance to glyphosate is evident as ‘present’ after the application of the herbicide. The plants remain alive after the application of the herbicide with no visible damage. Whereas, in non-GM other cotton varieties, herbicide tolerance is apparent as ‘absent’ due to the lack of the gene conferring tolerance. In those varieties the application of herbicide would kill the plants.

|     | English   | français  | Deutsch   | español  | Example Varieties | Note |
|-----|---|---|---|--|-------------------|------|
| (+) | <b>Plant: <u>glyphosate herbicide tolerance</u></b> | <b>Plante : tolérance <u>au glyphosate aux herbicides</u></b> | <b>Pflanze: <u>Herbizid Glyphosat</u>toleranz</b> | <b>Planta: tolerancia <u>a los herbicidas al glifosato</u></b> |                   |      |
| QL  | absent  | absente   | fehlend   | ausente  | [...]             | 1    |
|     | present   | présente  | vorhanden   | presente   | [...]             | 9    |

4.2.2.2 ~~Currently, a new A trait type of GM technology has been developed to provide both vegetative and reproductive tolerance to glyphosate. This technology uses the same gene but with a different promoter sequence which confers tolerance at both vegetative and reproductive stage. This trait is manifested as pollen: viability ‘present’ or in GM cotton varieties and ‘absent’ in non GM cotton varieties. In many cases, the GM and non GM varieties are morphologically indistinguishable. The only way to differentiate between the varieties is achieved with the application of herbicide.~~

|           | English                  | français                  | Deutsch                      | español                  | Example Varieties | Note |
|-----------|--------------------------|---------------------------|------------------------------|--------------------------|-------------------|------|
|           | <b>Pollen: viability</b> | <b>Pollen : viabilité</b> | <b>Pollen: Keimfähigkeit</b> | <b>Polen: viabilidad</b> |                   |      |
| (+)       |                          |                           |                              |                          |                   |      |
| <b>QL</b> | absent                   | absente                   | fehlend                      | ausente                  | [...]             | 1    |
|           | present                  | présente                  | vorhanden                    | presente                 | [...]             | 9    |

### 4.3 Plant Growth Regulators

4.3.1 Chemicals which act as plant growth regulators are often structurally similar to plant hormones. However, the basic difference between plant growth regulators and plant hormones is that growth regulators are exogenous (not made within the plant) whereas plant hormones are produced within the plants *per se* as a part of the biological process.

4.3.2 Plant growth regulators are commonly used to control plant height, lateral branching, flowering etc. Plant growth regulators (e.g. growth retardants) can simultaneously modify many plant characteristics and significantly alter the phenotype of a plant variety, e.g. the use of gibberellic acid (GA<sub>3</sub>) in the production of ‘Thompson Seedless’ grapes. These seedless grapes are widely used as a premium table grape. ‘Thompson Seedless’ grapes are produced as the result of GA<sub>3</sub> treatment of the grape variety named ‘Sultana’ (or ‘Sultania’), which is commonly used for the dry fruit market as raisins. However, when the variety ‘Sultana’ is treated with GA<sub>3</sub> (20-40ppm) at the early stage of fruit development the resulting fruits tend to elongate and the size of the fruit also increase and the fruits are then marketed as table grapes under the name ‘Thompson Seedless’.

4.3.3 Responses to a plant growth regulators could, in certain circumstances, be used as a characteristic if the requirements set out in Sections I.1.2 and 1.3 are met. However, where this is not the case, it may be difficult to ensure that the use of plant growth regulators in a DUS trial would not distort the DUS examination (see Section I.1.1). In particular, it would be difficult to ensure that a plant growth regulator would have an “equal effect” on all varieties included in the DUS test, including varieties of common knowledge. Furthermore, as plant growth regulators may have subtle effects on a range of plant characteristics, special care would be needed to ensure that the description of ‘standard characteristics’ included in the Test Guidelines were not distorted.



4.4 Explanations for chemical response characteristics in Test Guidelines

Where chemical response characteristics are included in Test Guidelines, the following information should be provided in Chapter 8 “Explanations on the Table of Characteristics”:

- (a) nature of the genetic control;
- (b) information on the chemical;
- (c) source(s) of chemical;
- (d) test method;
- (e) scoring procedure for determination of states of expression (notes); and
- (f) example varieties.

~~{5. — Frost tolerance}~~<sup>0</sup>

~~(to be considered)~~

## **SECTION II. CHEMICAL CONSTITUENTS: PROTEIN ELECTROPHORESIS**

1. The General Introduction (Section 4.6.2) states that “Characteristics based on chemical constituents may be accepted provided they fulfill the criteria specified in [the General Introduction] Section 4.2. It is important for those characteristics to be well defined and an appropriate method established for examination. More details can be found in document TGP/12, ‘Special Characteristics’.”

2. With regard to protein characteristics derived by using electrophoresis, UPOV has decided to place these characteristics in an annex to the Test Guidelines, thereby creating a special category of characteristic, because the majority of the members of the Union is of the view that it is not possible to establish distinctness solely on the basis of a difference found in a characteristic derived by using electrophoresis. Such characteristics should therefore only be used as a complement to other differences in morphological or physiological characteristics. UPOV reconfirms that these characteristics are considered useful but that they might not be sufficient on their own to establish distinctness. They should not be used as a routine characteristic but at the request or with the agreement of the applicant of the candidate variety.

3. For protein characteristics derived by using electrophoresis to be included in an annex to the Test Guidelines, it is necessary:

- (a) to establish the genetic control of the protein(s) concerned; and
- (b) to specify an appropriate method for the examination.

## **SECTION III. EXAMINATION OF CHARACTERISTICS USING IMAGE ANALYSIS**

### **1. Introduction**

Characteristics which may be examined by image analysis should also be able to be examined by visual observation and/or manual measurement, as appropriate. Explanations for observing such characteristics, including where appropriate explanations in Test Guidelines, should ensure that the characteristic is explained in terms which would enable the characteristic to be understood and examined by all DUS experts.

### **2. Combined characteristics**

2.1 The General Introduction (document TG/1/3, Chapter 4, Section 4) states that:

#### **4.6.3 Combined Characteristics**

4.6.3.1 A combined characteristic is a simple combination of a small number of characteristics. Provided the combination is biologically meaningful, characteristics that are assessed separately may subsequently be combined, for example the ratio of length to width, to produce such a combined characteristic. Combined characteristics must be examined for distinctness, uniformity and stability to the same extent as other characteristics. In some cases, these combined characteristics are examined by means of techniques, such as Image Analysis. In these cases, the methods for appropriate examination of DUS are specified in document TGP/12, 'Special Characteristics'.

2.2 Thus, the General Introduction clarifies that the use of image analysis is one possible method for examining characteristics which fulfil the basic requirements for use in DUS testing (see document TG/1/3, Chapter 4.2), which includes the need for the uniformity and stability of such characteristics to be examined. With regard to combined characteristics, the General Introduction also explains that such characteristics should be biologically meaningful.

### **3. Guidance on the use of image analysis**

[to be developed by the Technical Working Party on Automation and Computer Programs (TWC)]

|                       |         |  |
|-----------------------|---------|--|
| <u>Abbreviations:</u> | 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                         |
|                       | TWPs:   | Technical Working Parties                                      |

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- <sup>a</sup> The TWA, at its thirty-seventh session, held in Nelspruit, South Africa, from July 14 to 18, 2008, agreed that the title of document TGP/12 should be amended to remove reference to “special” characteristics, e.g. to rename as “Characteristics based on a response to an external factor and characteristics for chemical constituents: protein electrophoresis”. The CAJ, at its fifty-eighth session, held in Geneva on October 27 and 28, 2008, agreed that the new title, proposed by the TWA, “Characteristics based on a response to an external factor and characteristics for chemical constituents: protein electrophoresis” was too long and proposed that a shorter, clearer title should be found. The proposed title is based on a suggestion made by Mr. Chris Barnaby, Chairman of the TC. (Note: there is no reference to “physiological characteristics” in the General Introduction, nor in the document TGP/7/1 “Development of Test Guidelines”.)
- <sup>b</sup> The TWA proposed that “living organism” should be written in full throughout the document.
- <sup>c</sup> Proposal by Office of the Union (after TC-EDC meeting in January 2009) to update text in accordance with amendment to Section 1.2.2.1.
- <sup>d</sup> TC-EDC proposal at its meeting on January 8, 2009.
- <sup>e</sup> The TC, at its forty-fourth session, held in Geneva from April 7 to 9, 2008, agreed to invite the TWPs, in particular the TWV, to review the sentence “In general, for DUS purposes, ‘tolerance’ is not a suitable characteristic in relation to biotic factors.” and to modify the sentence to read “In many instances, for DUS purposes, tolerance may not be a suitable characteristic.”. As a part of the review, to consider the definition of “tolerance” for biotic factors and to consider whether it would be appropriate to explain why, in most instances, it is not used as DUS characteristic. A definition of tolerance for biotic factors was developed by Mr. Kees van Ettekovén (Netherlands), the drafter of Section I, 2 “Disease resistance”, and was agreed by the TWPs at their sessions in 2008. At its fifty-eighth session, held on October 27 and 28, 2008, the CAJ heard that the International Seed Federation (ISF) had concerns about the sentence “Effects should be related to yield reduction” and agreed that the ISF concerns should be reported to the expert responsible for drafting that text (Mr. van Ettekovén), in order that an amended wording might be developed for consideration by the TC. The amended wording is shown in double strikethrough for deletions and double underlining for additions.
- <sup>f</sup> Note by Office of the Union concerning discussions of BMT Guidelines which are also relevant for document TGP/12: At the seventy-fourth session of the Consultative Committee, held in Geneva on October 24, 2007, the Delegation of the United States of America wondered what the status of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add., would be on the adoption of the BMT Guidelines. In particular, it noted that the BMT Guidelines would be adopted by the Council, whereas documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5Add. had not been adopted by the Council. The Consultative Committee recommended that consideration be given to the status of documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5Add. with regard to their reference in the introduction of document BMT Guidelines (proj.9).
- The TC-EDC agreed that a solution would be to delete Section 1.3 of document TGP/12, on the basis that Option 1(a) was not restricted to characteristics included in TGP/12 and that documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. were planned to be revised. The TC-EDC also noted a question raised at the meeting as to whether documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. might be considered to be approved by the Council when it “noted the work of the Technical Committee, the Technical Working Parties and the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular, as given in document C/36/10 [“Progress report of the work of the Technical Committee, the Technical Working Parties and the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular”]” (see document C/36/13 “Report”, paragraph 21). The TC-EDC agreed that it would be a matter for the Consultative Committee to consider that question.

- <sup>g</sup> The TC-EDC proposed the deletion of Section 2.2.4.3 on the basis that the accepted UPOV definitions are provided in Section 1.2.2 and may diverge from the ISF definitions, e.g. see amendment in Section 1.2.2.1. (Note: the link provided by ISF does not contain definitions – it is the “Recommended Codes for Pest Organisms in Cereal and Vegetable Crops”.)
- <sup>h</sup> New weblink provided by International Seed Federation (ISF) (April 8, 2008).
- <sup>i</sup> Amended wording proposed by the TWA.
- <sup>j</sup> The TWA proposed that the sentence should be amended to also refer to cross-pollinated varieties.
- <sup>k</sup> Amended wording proposed by the TWA.
- <sup>l</sup> Amendment proposed by Office of the Union to correspond to the proposed change to Section 2.3.2.3.
- <sup>m</sup> The TC-EDC agreed to review if this paragraph is necessary.
- <sup>n</sup> Amendment proposed by the Office of the Union and agreed by Mr. Tanvir Hossain (Australia), drafter of Section 4 “Chemical Response”.
- <sup>o</sup> At its fortieth session, held in Kunming, China, from July 2 to 6, 2007, the TWO agreed to propose that consideration be given to including frost tolerance in the document. The TWF, at its thirty-eighth session, held in Jeju, Republic of Korea, from July 9 to 13, 2007, proposed to first check whether frost tolerance had been used as a DUS characteristic. At its forty-first session, held in 2008, the TWO heard that a characteristic for frost tolerance had been investigated by the European Community but had not resulted in distinctness. The TWO agreed that the section on frost tolerance should be deleted from TGP/12. The TWV agreed with the TWO conclusion that the section on frost tolerance should be deleted from TGP/12.
- <sup>p</sup> The TWA proposed to remove Section III: “Examination of characteristics using image analysis” from TGP/12 and include in document TGP/8, on the basis that it does not concern characteristics, but methods of examining characteristics. The TWC agreed with that proposal.

[End of document]