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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/12

## "SPECIAL CHARACTERISTICS"

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Note for Draft version

**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

TABLE OF CONTENTS PAGE

**SECTION I. DEVELOPMENT OF CHARACTERISTICS BASED ON A RESPONSE TO AN EXTERNAL FACTOR ..... 3**

1. INTRODUCTION ..... 3
*1.1 Requirements for characteristics based on a response to an external factor ..... 3*
*1.2 Terms Describing the Response of Plants to Pests, Pathogens or Abiotic Stresses Terminology in Disease Resistance (Definition of the Terms Describing the Reaction of Plants to Pests or Pathogens and to Abiotic Stresses) ..... 6*
1.2.1 Preamble ..... 6
1.2.2 Definitions ..... 6
1.2.2.1 Biotic factors (pest or pathogen) ..... 6
1.2.2.2 Abiotic factors (e.g. chemical, temperature) ..... 6
*1.3 Possible use of gene-specific molecular markers as predictors of traditional characteristics ..... 7*
2. DISEASE RESISTANCE ..... 8
*2.1 Introduction ..... 8*
*2.2 Criteria for use of disease resistance characteristics ..... 8*
2.2.1 Results from a given genotype or combination of genotypes (see Table 1 (a)) ..... 8
2.2.2 Is sufficiently consistent and repeatable in a particular environment (see Table 1(b)) ..... 8
2.2.3 Exhibits sufficient variation between varieties to be able to establish distinctness (see Table 1 (c)) ..... 8
2.2.4 Is capable of precise definition and recognition (see Table 1 (d)) ..... 8
2.2.5 Allows uniformity requirements to be fulfilled (see Table 1 (e)) ..... 9
2.2.6 Additional points for consideration ..... 9
(i) the availability of reliable inoculum and host differential set ..... 9
(ii) quarantine regulations ..... 9
(iii) the costs involved in disease resistance testing ..... 10
2.2.7 Information to be provided in Test Guidelines ..... 10
*2.3 Developing characteristics for disease resistance ..... 10*
2.3.1 Qualitative characteristics ..... 10
2.3.2 Quantitative characteristics ..... 11
*2.4 Explanations for disease resistance characteristics in Test Guidelines ..... 15*
3. INSECT RESISTANCE ..... 16
*3.1 Developing characteristics for disease resistance ..... 16*
*3.2 Example of Corn borer (Ostrinia nubilalis (Hübner)) resistance in maize varieties ..... 16*
*3.3 Example of resistance to Therioaphis maculate in Lucerne (UPOV Test Guidelines: TG/6/5) ..... 16*
*3.4 Example of resistance to colonization by Aphis gossypii in Melon ((UPOV Test Guidelines: TG/104/5) 17*
*3.5 Explanations for insect resistance characteristics in Test Guidelines ..... 18*
4. CHEMICAL RESPONSE ..... 19
*4.1 Introduction ..... 19*
*4.2 Herbicides ..... 19*
4.2.1 Herbicide Tolerant Varieties ..... 19
4.2.2 Case Study on the Use of Herbicide Tolerance as a Characteristic in the DUS Examination ..... 19
*4.3 Plant Growth Regulators ..... 20*
*4.4 Explanations for chemical response characteristics in Test Guidelines ..... 21*
[5. FROST TOLERANCE] ..... 21

**SECTION II. CHEMICAL CONSTITUENTS: PROTEIN ELECTROPHORESIS ..... 22**

**SECTION III. EXAMINATION OF ~~COMBINED~~ CHARACTERISTICS USING IMAGE ANALYSIS ..... 23**

1. INTRODUCTION ..... 23
2. COMBINED CHARACTERISTICS ..... 23
3. GUIDANCE ON THE USE OF IMAGE ANALYSIS ..... 23

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

### [a]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.

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

- the effect of factors, such as temperature, relative humidity and light, on the development or the agressivity of the L.O.
- the genetic variability of the L.O. (different pathotypes[1]).

[1] 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

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.

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 2 to 4 provide 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

| **Basic requirements that a characteristic should fulfill (document TG/1/3 Chapter 4, Section 4.6.1)** | **Particular considerations with regard to characteristics based on response to external factors** |
|---|---|
| *The basic requirements that a characteristic should fulfill before it is used for DUS testing or producing a variety description are that its expression:* | |
| *(a) results from a given genotype or combination of genotypes;* | knowledge of the nature of genetic control of the response is important |
| *(b) is sufficiently consistent and repeatable in a particular environment;* | (i) important to standardize, as far as possible, the conditions in the field, greenhouse or laboratory, as appropriate, and the methodology used;<br>(ii) the methodology should be validated, e.g. by a ring test; and<br>(iii) the key requirements should be set out in a protocol. |
| *(c) exhibits sufficient variation between varieties to be able to establish distinctness;* | the response and suitable states of expression should be described (see (d) below) |
| *(d) is capable of precise definition and recognition;* | (i) the external factor should be clearly defined and characterized (e.g. disease inoculum, fungal pathotype[2], virus pathotype [c], insect biotype, chemical etc.);<br>(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.<br>In general, for DUS purposes, "tolerance" is not a suitable characteristic in relation to disease resistance. |
| *(e) allows uniformity requirements to be fulfilled;* | 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. |
| *(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.* | the stability requirements for characteristics based on the response to external factors are the same as for other characteristics. |

[2] 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

[d]1.2 Terms Describing the Response of Plants to Pests, Pathogens or Abiotic Stresses Terminology in Disease Resistance (Definition of the Terms Describing the Reaction of Plants to Pests or Pathogens and to Abiotic Stresses)

*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 pathogen pathotypes 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.

In general, for DUS purposes, “tolerance” is not a suitable characteristic in relation to biotic factors.[e]

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 pose 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))*

[g]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 the consistency and repeatability of disease resistance, provided this is established for a particular pathotype, can be very good. [h] In fact, as disease resistance is of crucial importance for the marketing of varieties, it is a primary selection criteria for companies to check the varietal stability.

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

Disease resistance characteristics, if properly tested, can give a clear differentiation[j] in the variety collections. Therefore disease resistance characteristics are often used as grouping characteristics. The differentiation usually may take place even on pathotype level as many collections of varieties are known to show different resistance reactions to different pathotypes of the disease. Also on pathotype level grouping may be done, provided the pathotypes are properly identified. Guidance on the development of disease resistance as a qualitative or quantitative characteristic is provided in Section 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).

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.[k] 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 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/phytosanitary.htm).

2.2.4.4 Following the provided explanations in the test protocols, ring tests have shown to give deviating results. These deviations were caused by variation in the climatic conditions under which the trials were carried out. Also different interpretation of the symptoms by different observers was noted. The conclusion of these trials was that only if a correct set of standards was included in the trial, the observations and evaluation of the results was harmonized. It was however observed that slight differences in the standards (between lot differences) could cause problems. The advise here is to develop a centralized set of standards per disease or per pathotype to avoid problems. The seed industry is 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))*

Testing for disease resistance characteristics means introducing more variables in the trial; not only the development of the plants is subject to the environment, but also the quality of the inoculum, the inoculation and the interaction between symptom and development of the plant may cause variation within the trial. It has to be avoided that the heterogeneity introduced through the trial is blamed to the candidate variety.

*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 are maintaining[l] stocks of inoculum of most of the diseases that are used in breeding programs. In the explanation of the methods in the guidelines, the available information on these sources will have to be indicated. If inoculum from another source is used, a defined host differential set will have to be used to clearly identify the inoculum.

(ii) quarantine regulations

With a worldwide organization as UPOV, it is unavoidable that diseases that are of importance in a certain area, are unknown to cause problems in another part of the world and are there considered as quarantine diseases. Usually this means that the import of inoculum and the test itself is not possible. A good way to solve this kind of problems is to contact a DUS test authority elsewhere and ask them to carry out the test.

(iii) the costs involved in disease resistance testing

The costs and technical requirements of disease tests are for some DUS testing authorities impassable barriers to carry out these tests. Two options may be considered to overcome these problems:

- Another DUS testing authority may be asked to perform the necessary disease test(s).

- The applicant / breeder may be requested to carry out a blind disease test with coded samples including the candidate variety and a number of also coded control samples as susceptible and resistant controls on the basis of a clear control.

*2.2.7 Information to be provided in Test Guidelines*

In order to take into account the given points of consideration, the explanation of the disease resistance characteristics, included in the guidelines have to be extended with the necessary information on

- the address(es) where inoculum may be obtained,
- the host differential set of varieties / lines to use to check the inoculum on correctness regarding the pathotypes used,
- the address(es) where the differential set may be obtained
- the pathotype specific standard varieties to be included in the test
- the address(es) where the set of standard varieties may be obtained

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.[m]

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

| | English | français | Deutsch | español | Example Varieties | Note |
|---|---|---|---|---|---|---|
| **39. (+)** | **Resistance to downy mildew (*Bremia lactucae*)** | **Résistance au mildiou (*Bremia lactucae*)** | **Resistenz gegen Falschen Mehltau (*Bremia lactucae*)** | **Resistencia al mildiú (*Bremia lactucae*)** | | |
| **39.1** | **Isolate Bl 2** | **Isolat Bl 2** | **Isolat Bl 2** | **Aislado Bl 2** | | |
| **QL** | 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.[n] 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 |
|---|---|---|---|---|---|---|---|
| **70. (+)** | **VG** | **Resistance to *Sphaerotheca fuliginea (Podosphaera xanthii)* (Powdery mildew)** | **Résistance à *Sphaerotheca fuliginea (Podosphaera xanthii)* (oïdium)** | **Resistenz gegen *Sphaerotheca fuliginea (Podosphaera xanthii* (Echter Mehltau)** | **Resistencia a *Sphaerotheca fuliginea (Podosphaera xanthii)* (Oidio)** | | |
| **70.1** | | **Race 1** | **Pathotype 1** | **Pathotyp 1** | **Raza 1** | | |
| **QN** | | 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 The "1-3" scale recognizes that, for vegetatively propagated and self-pollinated varieties (see document TGP/9, Sections 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. 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.

[o]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 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 |
|---|---|---|---|---|---|---|---|
| **19. (+)** | **VS C** | **Resistance to *Colletotrichum trifolii*** | **Résistance à *Colletotrichum trifolii*** | **Resistenz gegen *Colletotrichum trifolii*** | **Resistencia al *Colletotrichum trifolii*** | | |
| **QN** | | 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 |

Example: Resistance to *Peronospora farinosa* f. *spinaciae* in Spinach (UPOV Test Guidelines TG/55/6) [p]

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **17. (+)** | | **Resistance to *Peronospora farinose* f. *spinaciae*** | **Résistance à *Peronospora farinosa* f. *spinaciae*** | **Resistenz gegen *Peronospora farinosa* f. *spinaciae*** | **Resistencia a *Peronospora farinosa* f. *spinaciae*** | | |
| **QL** | **VG** | | | | | | |
| **17.1** | | **Race 1** | **Race 1** | **Pathotyp 1** | **Raza 1** | | |
| | | absent | absente | fehlend | ausente | […] | 1 |
| | | present | présente | vorhanden | presente | […] | 9 |
| | | ---------- | ---------- | ---------- | ---------- | - | ---- |
| **17.2** | | **Race 2** | **Race 2** | **Pathotyp 2** | **Raza 2** | | |
| | | absent | absente | fehlend | ausente | […] | 1 |
| | | present | présente | vorhanden | presente | […] | 9 |
| | | ---------- | ---------- | ---------- | ---------- | - | ---- |

| | | | | | | |
|---|---|---|---|---|---|---|
| **17.3** | **Race 3** | **Race 3** | **Pathotyp 3** | **Raza 3** | | |
| | absent | absente | fehlend | ausente | […] | 1 |
| | present | présente | vorhanden | presente | […] | 9 |
| | ---------- | ---------- | ---------- | ---------- | - | ---- |
| **17.4** | **Race 4** | **Race 4** | **Pathotyp 4** | **Raza 4** | | |
| | absent | absente | fehlend | ausente | […] | 1 |
| | present | présente | vorhanden | presente | […] | 9 |

Ad. 17: Resistance to *Peronospora farinosa* f. *spinaciae*

Maintenance of races

Type of medium: Living host plants, obtainable from IPO-DLO, Wageningen, Netherlands

Special conditions: Propagation of separate races on living host plants, inoculation eleven days after sowing, following propagation cycle seven days after first.

In Scheme:

Day 0: sowing for first propagation
Day 7: sowing for second propagation
Day 11: inoculation first propagation
Day 14: sowing of third propagation
Day 18: inoculation second propagation
etc.

Number of host plants and propagations according to needs.
Resistance controls are included in the propagation cycle.

Execution of test:

Growth stage of plants: First cotyledons/leaf, eleven day old plants.

Temperature: 15°C during day/12°C during night.

Light: 15 hours per day, after emergence.

Growing method: Host plants and test plants are grown on modules of pot soil in glasshouse.

Method of inoculation: The infected leaves, taken from host plants that were infected seven days before, are washed in as little water as possible (maximum 150 ml water per 224 plants).

This suspension is filtered through cheese cloth. With 150 ml of suspension a maximum of 3 x 224 plants are infected. Spore density is 20.000 to 100.000 conidia/ml water. Suspension must be immediately sprayed over the test plants to ensure the vigor of the conidia. The leaves from the test plant should be wet, but no suspension should be dripping from the soil.

Remarks: Test is carried out in wintertime and protected against direct sunshine. After inoculation, the plants remain for three days under plastic, after this during daytime the plastic is slightly lifted.

Duration of test:

- Sowing to inoculation: 11 days.
- Inoculation to reading: 10 days.

Number of plants tested: 56 plants.

Evaluation of infection: Sporangia can be found on the lower side and later on the upper side of the leaves of susceptible plants.

Control varieties to identify races:

| | | | |
|---|---|---|---|
| race 1 | Susceptible | | Viroflay, Winterreuzen |
| | Resistant | | Condor |
| | | | |
| race 2 | Susceptible | | Master, Medania, Mega |
| | Resistant | | Trio, Spokane |
| | | | |
| race 3 | Susceptible | | Subito, Resistoflay |
| | Resistant | | Trio, Spokane |
| | | | |
| race 4 | Susceptible | | Trio, Spokane |
| | Resistant | | Chica, Ballet, Bolero |

2.4 Explanations for disease resistance characteristics in Test Guidelines[q]

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[r]

### 3.1 Developing characteristics for disease resistance

In general, insect resistance characteristics are qualitative or quantitative characteristics.

### 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.[s / t]

| | | English | français | Deutsch | español | Example Varieties | Note |
|---|---|---|---|---|---|---|---|
| **(+)** | **VG** | **Resistance to *Ostrinia Nubilalis Hübner*** | | | | | |
| **QN** | | susceptible | sensible | anfällig | susceptible | […] | 1 |
| | | present | présente | vorhanden | presente | […] | 9 |

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

In some cross-pollinated agricultural species (eg. Lucerne) insect resistance (eg. *Therioaphis maculata)* is often assessed as 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 |
|---|---|---|---|---|---|---|---|
| **22. (+)** | **VS C** | **Resistance to *Therioaphis maculata*** | **Résistance à *Therioaphis maculata*** | **Resistenz gegen *Therioaphis maculata*** | **Resistencia al *Therioaphis maculata*** | | |
| **QN** | | 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) [v]

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

Ad. 72: Resistance to colonization by *Aphis gossypii*

Maintenance of strain

| | |
|---|---|
| Maintenance and multiplication: | on susceptible variety (Vedrantais) |
| Special conditions: | weak greenfly density so as not to have too many winged types. "Synchronous"-type breeding so as to have only greenflies of the same age and therefore at the same growing stage on a plant |

Conduct of the test

| | |
|---|---|
| Plant stage: | 1st leaf measuring 2-3 cm |
| Temperature: | 21°C |
| Light: | 16 hours per day |
| Planting: | plants sown in sand, pricked out at cotyledon stage in compost-filled pots |
| Manner of inoculation: | deposit of ten adult wingless greenflies per plant |
| Duration of test: | |
| - from sowing to inoculation: | 15-18 days |
| - from inoculation to reading: | one day |
| Number of plants tested: | 30 |
| Recording: | - Resistance present = less than 7 adult aphids per plant; eggs rare.<br>- Resistance absent = 9 or 10 adult aphids per plant; eggs frequent.<br>- Record number of aphids per plant 24 hours after inoculation. |

3.5 Explanations for insect resistance characteristics in Test Guidelines[w]

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, defoliants, 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.

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 phenoxyaliphatic 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).

#### *4.2.2 Case Study on the Use of Herbicide Tolerance as a Characteristic in the DUS Examination*[x]

4.2.2.1 Herbicide tolerance which is discontinuously expressed as absent or present is a qualitative characteristic. In genetically modified cotton varieties, tolerance to glyphosate is evident as 'present' after the application of herbicide. The plants remain alive after the application of herbicide with no visible damage. Whereas, in non-GM 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. [y]

| | English | français | Deutsch | español | Example Varieties | Note |
|---|---|---|---|---|---|---|
| **(+)** | **Plant: herbicide tolerance** | | | | | |
| **QL** | absent | absente | fehlend | ausente | […] | 1 |
| | present | présente | vorhanden | presente | […] | 9 |

4.2.2.2 Currently, a new 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 is manifested as pollen: viability 'present' 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.[z]

| | English | français | Deutsch | español | Example Varieties | Note |
|---|---|---|---|---|---|---|
| | **Pollen: viability** | | | | | |
| **(+)** | | | | | | |
| **QL** | 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 (eg. growth retardants) can simultaneously modify many plant characteristics and significantly alter the phenotype of a plant variety, e.g. the use of gibberellic acid ($GA_3$) 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 $GA_3$ 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 $GA_3$ (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 plant growth regulators could, in certain circumstances, be used a characteristic if the requirements set out in Sections 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 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[aa]

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][bb]**

(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 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 ~~COMBINED~~[cc] 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

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. 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[dd]

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

*Notes*

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

[b] Moved to the Introduction of Section I and "[and that different genes lead to different genotypic expressions]" deleted (from draft 2), as requested by TWA and TWV (formerly Section 3.1).

[c] The TWA agreed that the TWV was the appropriate TWP to review the matter of whether the term "pathotype" was a suitable term to replace the terms race, strain etc.. The TWV agreed that the term "pathotype" could be used in TGP/12 to replace the terms "race", "strain" etc., although the terms "race", "strain" etc. should be used in the Test Guidelines where appropriate.

[d] Moved to the Introduction of Section I, as requested by TWA and TWV (formerly Section 2.3.2).

[e] The TWA and TWV proposed to explain as set out in Section I, Table 1(d) that, in general, for DUS purposes, "tolerance" is not a suitable characteristic in relation to biotic factors."

[f] Moved to the Introduction of Section I, as requested by TWA and TWV (formerly Section 2.5, 3.2.1).

[g] The TWA and TWV proposed to reverse the order of the sentences.

[h] The TWA and TWV proposed to edit the first sentence to be coherent with the terms used in the heading.

[i] The TWV proposed to correct the title in line with Table 1(c).

[j] The TWA and TWV proposed to amend the text as shown.

[k] The TWA and TWV proposed that the first sentence (in draft 2) should be deleted and second sentence to read as shown.

[l] The TWA and TWV proposed to delete "still" (in draft 2) in order to read as shown.

[m] The TWA and TWV proposed to read as shown.

[n] The TWA and TWV proposed to read as shown.

[o] TWA proposal: Mr. Tanvir Hossain (Australia), in conjunction with experts from Argentina, France and United Kingdom (the TGP/12 Section I subgroup), to prepare a draft subsection containing an example of a disease resistance characteristic for cross-pollinated varieties. Mr. Hossain to circulate a first draft to the members of the TGP/12 Section I subgroup by the end of June 2007, with their comments to be sent to Mr. Hossain by the end of July 2007. Mr. Hossain to then prepare a new draft for circulation to all TWPs by the end of August, with comments to be requested by the end of September, thus enabling a subsection to be included in TGP/12/1 Draft 3, to be considered by the Enlarged Editorial Committee in January 2008; the TWV agreed that Mr. Kees van Ettekoven (Netherlands) should be included in the TGP/12 Section I subgroup, as proposed by the TWA. With respect to the TWA proposal to prepare a draft subsection containing an example of a disease resistance characteristic for cross-pollinated varieties, the TWV agreed that Mr. van Ettekoven should propose a suitable example from a vegetable crop (e.g. Resistance to *Peronospora farinosa* f. *spinaciae* or to Cucumber mosaic virus (CMV) in Spinach). *Office note: the reason for the TWA proposing a "fast-track" process, of agreement by the TWPs by correspondence, was that there seemed to be a good level of agreement on TGP/12 and, therefore, a reasonable chance to finalize the document at the TC in April 2008. However, a number of further comments were made by the TWPs in 2007, indicating that it would not be appropriate to circulate a new draft for correspondence by the TWPs before the TC-EDC in January 2008.*

[p] With respect to the TWA proposal to prepare a draft subsection containing an example of a disease resistance characteristic for cross pollinated varieties, the TWV agreed that Mr. van Ettekoven should propose a suitable example from a vegetable crop (e.g. Resistance to Peronospora farinosa f. spinaciae or to Cucumber mosaic virus (CMV) in Spinach).

[q] The TWV proposed to provide guidance on the development of explanations for disease resistance characteristics, as required in Chapter 8 of the Test Guidelines, which could also be used a basis for similar guidance to be developed for Subsection 2 "Insect resistance" and Subsection 3 "Chemical response" through the work of the TGP/12 Section I subgroup.

[r] Introduction (in draft 2) moved to the Introduction of Section I, as proposed by TWA and TWV.

*Notes (continued)*

[s] The TWA and TWV proposed that, from " UPOV has also […]", to be moved to the Introduction of Section I and to delete "[and that different genes lead to different genotypic expressions]" (in draft 2).

[t] The TWA proposed sections 3.2.2.1 to 3.2.2.3 (in draft 2) to be condensed to the type of summary provided in Section 2.4 (in draft 2) and to present the characteristic with states of expression.

[u] The TWA proposed Mr. Hossain (Australia), in conjunction with the TGP/12 Section I subgroup, to prepare a new draft subsection containing an example for aphid resistance in cross-pollinated varieties.

[v] The TWV noted the TWA proposal for Mr. Hossain (Australia), in conjunction with the TGP/12 Section I subgroup, to prepare a new draft subsection containing an example for aphid resistance in cross-pollinated varieties. In that respect, the TWV proposed that Mr. van Ettekoven should propose an example from a vegetable crop (e.g. Resistance to colonization by Aphis gossypii in Melon).

[w] The TWV proposed to provide guidance on the development of explanations for disease resistance characteristics, as required in Chapter 8 of the Test Guidelines, which could also be used a basis for similar guidance to be developed for Subsection 2 "Insect resistance" and Subsection 3 "Chemical response" through the work of the TGP/12 Section I subgroup.

[x] Title amended, as proposed by TWA.

[y] The TWA proposed section to be condensed to the type of summary provided in Section 2.4 (in draft 2) and to present only the characteristic "Plant: herbicide tolerance" with the states of expression absent (1), present (9).

[z] The TWA proposed Mr. Hossain (Australia) to provide a new example within herbicide tolerance for a characteristic for pollen viability.

[aa] The TWV proposed to provide guidance on the development of explanations for disease resistance characteristics, as required in Chapter 8 of the Test Guidelines, which could also be used a basis for similar guidance to be developed for Subsection 2 "Insect resistance" and Subsection 3 "Chemical response" through the work of the TGP/12 Section I subgroup.

[bb] The TWO agreed to propose that consideration be given to including frost tolerance in the document. The TWF proposed to first check whether frost tolerance had been used as a DUS characteristic.

[cc] The TWC proposed that the text should be revised to consider simple characteristics before considering combined characteristics, because image analysis was most commonly used to observe simple characteristics.

[dd] The TWC discussed the possibility of seeking to develop general guidance on the use of image analysis and, in particular, the importance of comparing the results with human observations and the repeatability and reproducibility of the techniques. It also heard from the expert from Australia that freely available software had been used for image analysis in Australia and noted that it would be useful to include image analysis software in its discussions on exchangeable software. The TWC agreed to have an item on the agenda of its twenty-sixth session to consider those matters and to receive an update on the use of image analysis by UPOV members and to develop guidance on good practice.

[End of document]