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

### **1. Introduction**

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.”

1.2 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.

1.3 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.

1.4 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. <sup>a</sup>

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
<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>	
(a) <i>results from a given genotype or combination of genotypes;</i>	knowledge of the nature of genetic control of the response is important
(b) <i>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>
(c) <i>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)
(d) <i>is capable of precise definition and recognition;</i>	<p>(i) the external factor should be clearly defined and characterized (e.g. disease inoculum, fungal race, virus pathotype, insect biotype, chemical etc.)<sup>b</sup>;</p> <p>(ii) the type of response to the external factor (e.g. disease: susceptible / intermediate resistant / resistant; abiotic factors: sensitive / tolerant<sup>c</sup>, etc.)<sup>b</sup> 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>In general, for DUS purposes, “tolerance” is not a suitable characteristic in relation to disease resistance.<sup>d</sup></p>
(e) <i>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.
(f) <i>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.

## 2. Disease Resistance

### 2.1 Introduction

Resistance to pests and diseases is an important breeding aim, particularly in vegetable breeding. 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

~~2.2.1 As with other characteristics (see Section 1.3: Table and the General Introduction, Chapter 4.2.1), the basic requirements that a disease resistance 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;~~
- ~~(b) is sufficiently consistent and repeatable in a particular environment;~~
- ~~(c) exhibits sufficient variation between varieties to be able to establish distinctness;~~
- ~~(d) is capable of precise definition and recognition;~~
- ~~(e) allows uniformity requirements to be fulfilled;~~
- ~~(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.~~ <sup>e</sup>

In general, the requirements set out in Table 1 <sup>f</sup> 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))*

Cooperation with breeders also results in better knowledge on the genetic background of the various forms of disease resistance. 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.

#### 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 stability of disease resistance, provided this was established on race / strain level is very good. 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 *Is sufficiently consistent and repeatable in a particular environment (see Table 1 (c))*

Disease resistance characteristics, properly tested, give per definition a clear differentiation in the variety collections. Therefore disease resistance characteristics are often used as grouping characteristics. The differentiation usually may take place even on race / strain level as many

collections of varieties are known to show different resistance reactions to different races / strains of the disease. Also on race / strain level grouping may be done, provided the races / strains are properly identified. Guidance on the development of disease resistance as a qualitative or quantitative characteristic is provided in Section 2.4 [cross ref].<sup>g</sup> A specific problem are those diseases or races / strains of diseases, where the difference between susceptible and resistant is not discontinuous, but in fact a scale of resistance can be observed ranging from absent to very weak to very strong. In practice, however, it is not yet possible to define the different levels using example varieties, so in the guidelines diseases that show this phenomenon are usually treated as discontinuous by defining a threshold dividing susceptible from resistant. The threshold is clearly defined using example varieties. It may be expected that in future this practice will be replaced by a more precise description of the different levels of resistance. These levels have to be defined precisely and standards will have to be included in the tests to enable the differentiation between the different levels.

#### 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 definition and denomination of the races and strains per disease pose a specific, more complicated problem as almost no longer any scientific work is done on this subject. This can result in confusing situations where the same race / strain could be named differently in Europe and the USA e.g. *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomato where race 1 in the USA is identical to race 0 in Europe. Also different races / strains 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 races / strains 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 2.3 [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>).<sup>h</sup>

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 strain 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 still maintaining 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 races / strains used,

- the address(es) where the differential set may be obtained
- the race / strain specific standard varieties to be included in the test
- the address(es) where the set of standard varieties may be obtained

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

### 2.3.1 *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 races capable of overcoming resistance may emerge.

### 2.3.2 *Definitions<sup>j</sup>*

The following definitions are intended for the purpose of the examination of DUS:<sup>k</sup>

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

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

Resistance is 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.

~~The Vegetable Section of ISF recommends, as it pertains to biotic stress, that its members use the terms immunity, high/standard or moderate/intermediate resistance and susceptibility and to avoid the term tolerance in communications with their customers.<sup>l</sup>~~

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

~~Tolerance is the ability of a plant variety to endure abiotic stress without serious consequences for growth, appearance and yield. Vegetable companies will continue to use tolerance for abiotic stress. is the ability of a plant variety to endure biotic stress (including disease) or abiotic stress, without serious consequences for growth, appearance and yield.<sup>m</sup>~~

~~Sensitivity is the inability of a plant variety to endure biotic stress (including disease) or abiotic stress without serious consequences for growth, appearance and yield.<sup>n</sup>~~

## 2.4 Developing characteristics for disease resistance

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

### 2.4.1 *Qualitative characteristics*

Disease resistances which are expressed as absent or present, where those states are discontinuous, are qualitative characteristics.

#### Example

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

### 2.4.2 *Quantitative characteristics*

2.4.2.1 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 the 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

	English	français	Deutsch	español	Example Varieties	Note
<b>70.</b>	<b>VG</b>	<b>Résistance à</b>	<b>Resistenz gegen</b>	<b>Resistencia a</b>		
(+)	<i>Sphaerotheca fuliginea</i> ( <i>Podosphaera xanthii</i> ) (Powdery mildew)	<i>Sphaerotheca fuliginea</i> ( <i>Podosphaera xanthii</i> ) (oïdium)	<i>Sphaerotheca fuliginea</i> ( <i>Podosphaera xanthii</i> ) (Echter Mehltau)	<i>Sphaerotheca fuliginea</i> ( <i>Podosphaera xanthii</i> ) (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 resistant	moderadamente resistente	[...]	2
	highly resistant	hautement résistant	hochresistent	altamente resistente	[...]	3

2.4.2.2 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.<sup>o</sup>

### **3.<sup>p</sup> Insect Resistance**

#### **3.1 Introduction**

3.1.1 Among the characteristics which can be used to establish distinctness of a candidate variety, some are the result of the interaction between two living organisms: the plant variety and a fungus; a bacterium; a virus or an insect (designated as L.O. in this paper).

3.1.2 In such cases, certain specific conditions must be considered because of the possible variation of the L.O. which interacts with the variety.

3.1.3 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 aggressivity of the L.O.
- the genetic variability of the L.O. (different races or strains).

3.1.4 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.

3.1.5 Different situations must be considered. The<sup>q</sup> assessment of insect resistance based on a genetic modification in maize varieties is described as an example.

#### **3.2 Example: Corn borer resistance in GM<sup>r</sup> maize varieties**

##### **3.2.1 *Methods***

The following example concerns a procedure for corn borer resistance in maize varieties where the resistance has been introduced by genetic modification. The procedure involves a bioassay approach. UPOV has also 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 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 corn borer resistance [and that different genes lead to different genotypic expressions].<sup>s</sup>

~~†The procedure can include two parts:~~

- ~~(a) — Check on the expression of the transgene: Bioassay~~
- ~~(b) — Check on the presence of the transgene~~

~~The strategy on how to use these two tests can be as follows:~~

### 3.2.1 *Check on the expression of the transgene: Bioassay*

3.2.1.1 The expression of the transgene is directly observed in a test where the plant and the insect interact using pieces of young leaves and corn borer larvae. The protocol is described in Section 3.2.3 [cross ref].

3.2.1.2 This test works well and it enables the efficiency of the genetic transformation to be assessed. Compared to a PCR test, or Elisa test, which only reveal the presence of the protein, the Bioassay brings information on the real effect on the insect.

3.2.1.3 The present experience is that the transgenes which have been developed up until now are efficient whatever the origin of the corn borer.

### 3.2.2 *Check on the presence of the transgene*

3.2.2.1 When sufficient experience has been gathered on a given transgenic event<sup>1</sup> and if no interaction has been observed on the expression of the transgene between the transgene and the plant genetic background, the test to check the corn borer resistance could be done using PCR technique.

3.2.2.2 It is assumed that the specific probe<sup>2</sup> is available to recognize the transgenic event.

3.2.2.3 Each time a new transgene is developed, its expression in different genetic backgrounds must be checked before relying on PCR technique alone to assess the characteristic.

3.2.2.4 It is also important to clarify that whatever the transgene or the transgenic event used, only one characteristic is considered to establish distinctness: corn borer resistance. It means that distinctness does not rely on differences in transgenes or transgenic events with the same expression.

### 3.2.2 *Protocol for the Bioassay to check Corn Borer (Ostrinia Nubilalis Hübner) Resistance of GM Maize Varieties*

3.2.2.1 The protocol is as follows:

- Plants in growth with 8 to 10 leaves
  - Larvae at the stage L1 (1<sup>st</sup> stage of development)
- (a) Pieces of leaves are sampled plant-by-plant on 10 plants per variety;
  - (b) Leaves of each plant are distributed in 5 water-tight plastic boxes of 45 mm of diameter in which a disk of watered filter paper has been placed;
  - (c) Six larvae are placed in each box; in total 50 boxes and 300 larvae per variety are used;

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<sup>1</sup>—transgenic event = a transgene transferred to a given location in the plant genome using an appropriate technique

<sup>2</sup>—Specific probe means a probe with which the identity of the transgenic event (the transgene and its location in the genome) can be precisely determined.

(d) A susceptible variety is always included in each bioassay.

#### 3.2.2.2 Conditions and Observations:

(e) The boxes are placed in a chamber at 25° C with a photophase 16 : 8 (16 hours of light and 8 hours of dark) during 4 days with saturated moisture;

(f) Mortality is recorded after 4 days exposure and surviving larvae are recorded on the 5<sup>th</sup> day.

#### 3.2.2.3 Expression of the results

(g) The criteria to assess resistance is the death rate of larvae;

(h) The total number of dead larvae per plant is recorded as a percentage;

(i) The average percentage per variety and a standard deviation are computed.

## 4. P 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<sup>u</sup> 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 Herbicides in the Expression of Plant Characteristics and Assessing Distinctness*

4.2.2.1 Glyphosate tolerance in genetically modified cotton varieties can be used as a study case of the way in which morphological characteristics expressed in response to a particular chemical compound can be used to assess distinctness.

4.2.2.2 It has been reported (Australian PBR trials, 2000-2004) that certain phenotypic characteristics with different states of expressions were noticeable when different cotton varieties were treated with commercial concentrations of glyphosate. An example of an “additional characteristic”<sup>3</sup> (Plant death) specially developed in cotton in response to the application of *glyphosate* is presented in Table 2:<sup>v</sup>

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<sup>3</sup> These characteristics are additional to the “standard characteristic” included in the UPOV Test Guidelines for a species and are often specifically developed for particular circumstances.

**Table 2: Example in cotton of responses to the application of *glyphosate* and the use of one response (Plant death) as a characteristic<sup>v</sup>**

<b>Response to Herbicide</b>	<b>Effects</b>	
Young leaf folding	very low effect	
	low effect	
	medium effect	
	strong effect	
	very strong effect	
Leaf blotching	very low effect	
	low effect	
	medium effect	
	strong effect	
	very strong effect	
Terminal chlorosis	very low effect	
	low effect	
	medium effect	
	strong effect	
	very strong effect	
Plant wilting	very low effect	
	low effect	
	medium effect	
	strong effect	
	very strong effect	
*Plant death	absent	Note 1
	present	Note 9

Note: The scores on leaf blotching, terminal chlorosis and plant wilt are taken both at 3 and 7 days after the treatment. The scores on young leaf folding are taken at 7 days after herbicide treatment. The scores on plant death are assessed 14 days after spraying.

\* = additional characteristic for the examination of DUS

4.2.2.3 Table 3 shows data on herbicide induced plant **responses** from a cotton trial in Australia that had been sprayed with *glyphosate* (using the additional characteristic included in Table 2)

Table 3: Comparison of cotton varieties on the basis of *glyphosate* tolerance<sup>v</sup>

RESPONSE TO HERBICIDE	Variety		
	'NuPearl RR'	'DP 5690 RRI'	'DeltaPEARL'
YOUNG LEAF FOLDING			
*DAS 7 mean	very low	very low	medium to strong
LEAF BLOTCHING			
DAS 3 mean	very low	very low	medium
DAS 7 mean	very low to low	very low to low	strong to very strong
TERMINAL CHLOROSIS			
DAS 3 mean	very low	very low	very low
DAS 7 mean	very low	very low	medium
PLANT WILT			
DAS 3 mean	very low	very low	very low to low
DAS 7 mean	very low	very low	medium
PLANT DEATH			
DAS 14 mean	Note 1	Note 1	Note 9
	(plants alive)	(plants alive)	(plants dead)

\* DAS = days after spraying; scoring was done at 3, 7 and 14 days after herbicide application (eg DAS 7 means 7 days after spraying).

4.2.2.4 The above data shows that, following *glyphosate* treatment, the differences between the tolerant and sensitive<sup>u</sup> varieties become evident within a week for all characteristics mentioned above. Both 'NuPearl RR' and 'DP 5690 RRI' varieties were tolerant to *glyphosate*, showing very little effect, while the variety 'DeltaPEARL' was completely sensitive<sup>u</sup> and was dead from the treatment by day 14. Without using the additional characteristic "Plant death"<sup>v</sup> it would be difficult to distinguish between those varieties, which are morphologically almost indistinguishable.

#### 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<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'.<sup>w</sup>

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.

## **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 CHARACTERISTICS USING IMAGE ANALYSIS**

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. 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.

## Notes

- <sup>a</sup> The TWO noted that the document is based upon examples and that general recommendations are provided only in Section 1. Therefore, it considered that it was necessary to expand those recommendations and to clarify that the examples are not an exclusive list of special characteristics.
- <sup>b</sup> Text proposed by Mr. Kees van Ettekoven (Netherlands), drafter of the section on disease resistance.
- <sup>c</sup> Proposed to be deleted, but necessary for herbicide tolerance (Section 4).
- <sup>d</sup> Text proposed by TWV, as modified by Mr. van Ettekoven.
- <sup>e</sup> Deletion proposed by TWA.
- <sup>f</sup> Revision of text as a consequence of the deletion of the preceding paragraph.
- <sup>g</sup> The TWA proposed to review the paragraph to reflect the fact that quantitative characteristics are accepted as shown in paragraph 2.4.2.
- <sup>h</sup> The TWA proposed to replace the reference to an annex with a reference to the ISF website.
- <sup>i</sup> Title of Section 2.3 amended according to proposed change of definition of “tolerance”.
- <sup>j</sup> The TC requested that the definitions provided in Section 2.3.2 should be compared with the definitions previously agreed by the TC, which are as follows (see document TC/32/7, paragraph 17(i)):

### “Definition of the terms describing the reaction of plants to pests and pathogens

- The definitions below concern exclusively the specific host-parasite pairs between which there exists compatibility. They do not concern non-recognition between partners amounting to incompatibility.
- There exist differing degrees of specificity in the host-parasite relations. The identification of that specificity generally requires the use of highly elaborate analytical means.
- Recognizing whether a plant is subject or not to parasites may depend on the analytical method.
- It is important, in general, to stress that the specificity of pests or pathogens may vary over time and space and that new pathogen races or new pest biotypes capable of overcoming a resistance may emerge.

### “The following terminology has been adopted by UPOV:

Resistance: The ability of a variety or of a mono-specific population to limit the activities of a given pest of pathogen throughout the whole or a part of a growing cycle. Several resistance levels may generally be defined.

Susceptibility: Susceptibility corresponds to a zero-resistance level of a variety or population with respect to a given pest or pathogen.

Tolerance: Ability of a variety or population to tolerate the development of a pest or pathogen whilst displaying disorders that are without serious consequences for their growth, appearance or yield.”

Mr. Kees van Ettekoven, drafter of the Section on disease resistance commented that “In particular the term tolerance is difficult to establish in DUS trials. As also in practice the use of the term tolerance is leading to interpretation difficulties, in this document it is proposed to accept the terminology for DUS purposes as presented in Section 2.3.2. Further the use of the proper states of expression is indicated.”

- <sup>k</sup> The TWA proposed to clarify that the definitions were intended for UPOV purposes only.
- <sup>l</sup> The TWV proposed that the paragraph before “Tolerance” be deleted.
- <sup>m</sup> Amended wording proposed by TWV, as modified by Mr. van Ettekoven.
- <sup>n</sup> The TWA proposed to explain that the term sensitivity is the opposite of tolerance.
- <sup>o</sup> The TWA proposed to make reference to the general requirement for two notes difference in quantitative characteristics for the establishment of distinctness, as set out in TGP/9, i.e. to clarify that only pairs of varieties which were susceptible (Note 1) and highly resistant (Note 3) could be considered distinct on the basis of Notes.
- <sup>p</sup> The TWA proposed “Insect Resistance” to be moved before “Chemical Response” in recognition of the fact that new Sections 2 and 3 would concern resistance, whereas Section 4 would concern tolerance.
- <sup>q</sup> To delete “In this first draft document”.
- <sup>r</sup> The TWA proposed to delete reference to “GM” in the title and provide a brief explanation of the development of corn borer resistance through genetic modification in the introduction.

*Notes (continued)*

<sup>s</sup> The TWA proposed that paragraphs up to 4.2.3 be deleted and replaced by reference to the situation in UPOV concerning the use of molecular techniques as set out in documents TC/38/14 -CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add., explaining in particular that only a bioassay approach had been developed and that an Option 1(a) approach would require that a reliable linkage between the presence of the transgene and the expression of corn borer resistance be established.

<sup>t</sup> The TWA proposed that the paragraphs up to 4.2.3 be deleted and replaced by reference to the situation in UPOV concerning the use of molecular techniques as set out in documents TC/38/14 -CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add., explaining in particular that only a bioassay approach had been developed and that an Option 1(a) approach would require that a reliable linkage between the presence of the transgene and the expression of corn borer resistance be established.

<sup>u</sup> The TWA proposed to replace “resistant” with “tolerant” and “susceptible” with “sensitive” in relation to herbicide effects.

<sup>v</sup> The TWA proposed to remove the attribution of Notes to herbicide effects, except in relation to plant death, and to clarify that effects other than plant death were not being used as DUS characteristics.

<sup>w</sup> The TWO proposed to reword the paragraph to clarify that ‘Thompson Seedless’ refers to the fruit obtained from variety ‘Sultania’. At the moment it gives the impression that by using growth regulators it may be possible to register two varieties when in fact there is only one variety.

[End of document]