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Associated Document
to the
General Introduction to the Examination
of Distinctness, Uniformity and Stability and the
Development of Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

DOCUMENT TGP/12

"GUIDANCE ON CERTAIN PHYSIOLOGICAL CHARACTERISTICS"

Document prepared by the Office of the Union

to be considered by

the Council at its forty-sixth ordinary session,
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SECTION I: DEVELOPMENT OF CHARACTERISTICS BASED ON A RESPONSE TO AN EXTERNAL FACTOR

1. Introduction

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

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

> "The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc. In some cases (e.g. disease resistance), reaction to certain factors is intentionally used (see TG/1/3 Chapter 4, Section 4.6.1) as a characteristic in the DUS examination. However, where the factor is not intended for DUS examination, it is important that its influence does not distort the DUS examination. Accordingly, depending on the circumstances, the testing authority should ensure either that:
>
> (a) the varieties under test are all free of such factors or,
>
> (b) that all varieties included in the DUS test, including varieties of common knowledge, are subject to the same factor and that it has an equal effect on all varieties or,
>
> (c) in cases where a satisfactory examination could still be undertaken, the affected characteristics are excluded from the DUS examination unless the true expression of the characteristic of the plant genotype can be determined, notwithstanding the presence of the factor."

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

1.1.3 In the case of external factors which are living organisms, certain specific conditions must be considered because of the possible variation of the living organism which interacts with the variety. In comparison with climatic or soil factors, additional sources of variation can change the effect of the living organism on the variety:

- the effect of factors, such as temperature, relative humidity and light, on the development or the aggressivity of the living organism

- the genetic variability of the living organism (different pathotypes[1]).

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

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[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.

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; (ii) the methodology should be validated, e.g. by a ring test; and (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[1], virus pathotype, insect biotype, chemical etc.); (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.
(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.

1.2 Terms Describing the Response of Plants to Pests, Pathogens and 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 types of 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.

Tolerance: is the ability of a plant to limit the negative effects of a specified pest or pathogen.

Effects should be related to aspects such as yield reduction.[2]

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

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 or yield.

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

2. Disease Resistance

2.1 Introduction

Resistance to pests and diseases poses particular challenges, in particular with regard to the precise definition and recognition of characteristics and ensuring sufficient consistency and repeatability. The following sections address those requirements and the other requirements that a characteristic is required to fulfill.

2.2 Criteria for use of disease resistance characteristics

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

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[2] In many instances, for DUS purposes, tolerance may not be a suitable characteristic because the method required to establish different levels of tolerance requires a method of examination beyond the usual scope of a DUS test in one place in a limited number of replicates.

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

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

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

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

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

Disease resistance characteristics, if properly tested, can give a clear differentiation in the variety collections. The differentiation may take place at the pathotype level because many collections of varieties are known to show different resistance reactions to different pathotypes of the disease. Guidance on the description of qualitative and quantitative disease resistance characteristics is provided in Section I, 2.3.

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

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

2.2.4.2 The same pathotype may be named differently in different parts of the world, e.g. *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomato, where race 1 in the United States of America is identical to race 0 in Europe. Also, different pathotypes may have the same name, e.g. *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomato, where race 2 in the USA is different from race 2 in Europe. At the moment a joint effort is made by International Seed Federation (ISF) on this subject with the aim to create one clear system of definition and nomination. The core of this system is the precise definition of a set of host differential lines/varieties with which the pathotypes can be determined. The seed industry is often willing to cooperate by maintaining the necessary stocks of seed for this purpose.

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

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

The development of inoculated plants is influenced by the environment and the quality of the inoculum. The inoculation method and the state of development of the plant may cause variation in symptoms developing in the plants within the trial. Such variation should not be assumed to be the result of a lack of uniformity of the variety (see document TGP/10/1, Section 4.6).

2.2.6 Additional points for consideration

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

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

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

(ii) quarantine regulations

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

(iii) technical requirements

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

2.3 Developing characteristics for disease resistance

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

2.3.1 Qualitative characteristics

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

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

	English	français	deutsch	español	Example Varieties	Note
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

Disease resistances for which there is a continuous range of levels of susceptibility / resistance across varieties, are quantitative characteristics. Guidance for the development of appropriate states of expressions for quantitative characteristics is provided in document TGP/9, Guidance Note GN 20, section 3.

Example with "1–3" scale: 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

Example with "1–9" scale: 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

2.4 Explanations for disease resistance characteristics in Test Guidelines

2.4.1 Where disease resistance characteristics are included in Test Guidelines, information should be provided in Chapter 8 "Explanations on the Table of Characteristics" in the form of a standard disease resistance test protocol as set out below. This standard resistance protocol is guidance and not a strict prescription. It is not only advised to use the subjects mentioned, it also is advised to use the same order of the subjects. In order to increase the legibility and use of the protocols it is also advised to restrict the number of extra topics. Compulsory elements are printed in bold, the other elements may be used depending on the resistance test protocol. (The elements in bold font should not be presented in bold font in the Test Guidelines.)

STANDARD RESISTANCE PROTOCOL

1. Pathogen
2. Quarantine status
3. Host species
4. Source of inoculum
5. Isolate
6. Establishment isolate identity
7. Establishment pathogenicity
8. Multiplication inoculum
   8.1 Multiplication medium
   8.2 Multiplication variety
   8.3 Plant stage at inoculation
   8.4 Inoculation medium
   8.5 Inoculation method
   8.6 Harvest of inoculum
   8.7 Check of harvested inoculum
   8.8 Shelflife/viability inoculum
9. Format of the test
   9.1 Number of plants per genotype
   9.2 Number of replicates
   9.3 Control varieties
   9.4 Test design
   9.5 Test facility
   9.6 Temperature
   9.7 Light
   9.8 Season
   9.9 Special measures
10. Inoculation
   10.1 Preparation inoculum
   10.2 Quantification inoculum
   10.3 Plant stage at inoculation
   10.4 Inoculation method
   10.5 First observation
   10.6 Second observation
   10.7 Final observations
11. Observations
   11.1 Method
   11.2 Observation scale
   11.3 Validation of test
   11.4 Off-types
12. Interpretation of data in terms of UPOV characteristic states
13. Critical control points

2.4.2 It is advised not to include all non compulsory elements in each Test Guidelines but rather to provide references to UPOV members that have experience with the relevant disease resistance protocol.

2.5 The nomenclature of pathogens

2.5.1 As in the plant kingdom, also in the field of pathogens the denomination of the subject is important in order to correctly identify the various diseases. The names of pathogens sometimes have to change as a consequence of improved insight in the pathogen and its relation with other pathogens. Continuous attention to the proper use of names is therefore important.

2.5.2 In the seed trade, because of limited space on seed labels, the scientific binomial for the pathogens is normally replaced by a code. In the disease resistance coding working group of the International Seed Federation (ISF) a system of codes was introduced to ensure uniformity in the use of these codes. The codes are derived from the names of the pathogens and can also be found on the ISF website: www.worldseed.org on the subject of pathogen coding. It is advised to introduce the disease codes in the Test Guidelines. The old name will keep the appropriate code, e.g. *Oidium neolycopersici* (ex *Oidium lycopersicum*) On (ex Ol).

2.5.3 It is also advised to use the same separators as used by ISF, for example :(colon) to separate the species code from the strain/race/pathotype code. The colon is followed by a space e.g. in Bl: 1-25.

3. Insect Resistance

3.1 Developing characteristics for insect resistance

The following examples of insect resistance characteristics are provided for illustrative purposes.

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

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

		English	français	deutsch	español	Example Varieties	Note
		Resistance to Ostrinia Nubilalis Hübner	**Résistance à Ostrinia Nubilalis Hübner**	**Resistenz gegen Ostrinia Nubilalis Hübner**	**Resistencia al Ostrinia Nubilalis Hübner**		
QL		absent	absente	fehlend	ausente	[…]	1
		present	présente	vorhanden	presente	[…]	9

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

In some cross-pollinated species (e.g. Lucerne) insect resistance (e.g. *Therioaphis maculata)* is assessed as the percentage of resistant plants within the population. In those cases a continuous range of variation could be observed 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)*

		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	absente	fehlend	ausente	[…]	1
		present	présente	vorhanden	presente	[…]	9

3.5 Explanations for insect resistance characteristics in Test Guidelines

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

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

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

4. Chemical Response

4.1 Introduction

Plant growth can be significantly influenced by a number of chemical compounds. When applied to plants, such chemicals can affect the phenology, physiology and change phenotypic characteristics. They include herbicides, plant growth regulators, 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

When herbicide tolerant 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.

4.2.2 Case Study on the Use of Herbicide Tolerance in Cotton as a Characteristic in the DUS Examination

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

	English	français	deutsch	español	Example Varieties	Note
(+)	**Plant: glyphosate tolerance**	**Plante : tolérance au glyphosate**	**Pflanze: Glyphosattoleranz**	**Planta: tolerancia al glifosato**		
QL	absent	absente	fehlend	ausente	[…]	1
	present	présente	vorhanden	presente	[…]	9

4.2.2.2 In addition to situations where the glyphosate tolerance relates to the "whole plant", situations can arise where only particular organs express tolerance. For example, a trait has been developed to allow the pollen of otherwise glyphosate-sensitive cotton varieties to remain viable following the application of the herbicide. The following characteristic is an example of a characteristic developed on the basis of that trait:

	English	français	deutsch	español	Example Varieties	Note
(+)	**Pollen: viability after glyphosate application**	**Pollen: viabilité après application de glyphosate**	**Pollen: Lebens-fähigkeit nach Anwendung von Glyphosat**	**Pollen: viabilité après application de glyphosate**		
QL	absent	absente	fehlend	ausente	[…]	1
	present	présente	vorhanden	presente	[…]	9

4.3 Plant Growth Regulators

Response to a plant growth regulator could, in certain circumstances, be used as a characteristic if the requirements set out in Section I, 1.1.2 and 1.1.4 are met. However, where this is not the case, it may be difficult to ensure that the use of plant growth regulators in a DUS trial would not distort the DUS examination (see Section I, 1.1). In particular, it would be difficult to ensure that a plant growth regulator would have an "equal effect" on all varieties included in the DUS test, including varieties of common knowledge. Furthermore, as plant growth regulators may have subtle effects on a range of plant characteristics, special care would be needed to ensure that the description of 'standard characteristics' included in the Test Guidelines were not distorted.

4.4 Explanations for chemical response characteristics in Test Guidelines

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

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

SECTION II. CHEMICAL CONSTITUENTS: PROTEIN ELECTROPHORESIS

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

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

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

(a) to establish the genetic control of the protein(s) concerned; and

(b) to specify an appropriate method for the examination.

4. Examples of protein characteristics derived by using electrophoresis can be found in the Test Guidelines for Barley (document TG/19/10), for Maize (document TG/2/7) and for Wheat (document TG/3/11 + Corr.).

[End of document]