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International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables

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ASSESSING DISTINCTNESS IN DISEASE RESISTANCE CHARACTERISTICS

Document prepared by experts from France and the Netherlands, with the support of European Union, Japan and breeders' organizations

Disclaimer: this document does not represent UPOV policies or guidance

EXECUTIVE SUMMARY

1. There are certain quantitative (QN) disease resistance characteristics where it is not possible to describe different levels of resistance according to the current QN states of expression. These levels could be caused by few genes with overlapping levels of resistance and are influenced by testing conditions. The genetic background might not be completely known. In this case, only two levels of resistance can describe the distinctiveness: "absent or low" (1) on one side and "medium or high" (2) on the other side.

2. Therefore, there is a need to introduce a new type of expression of characteristics, or at least to introduce amendments to the definition of the QN type of expression of characteristics.

BACKGROUND

3. The TWV, at its fifty-seventh session (May 2023),

- Received a contribution from the experts from France (FR) and the Netherlands (Kingdom of) (NL) (TWV/57/10) to propose the base of a guidance explaining the particular features of disease resistance characteristics that require special treatment in relation to general UPOV guidance.
- The draft guidance would allow to establish distinctness for quantitative disease resistance characteristics based on a difference of one note between varieties.

- In its report, (see document TWV/57/26 CORR. "Report", paragraphs 23 to 24), the purpose of additional discussion was defined.

> 23. The TWV agreed there were certain quantitative (QN) disease resistance characteristics where it was not possible to describe different levels of resistance according to QN states of expression because of the influence of testing conditions and the lack of information on genetic background.
>
> 24. The TWV agreed to invite the experts from France and the Netherlands, with the support of the European Union, Japan and the breeders' organizations, to draft a proposal for a special type of quantitative disease resistance characteristic with only two states of expression. The TWV agreed that the proposal with an explanation on the criteria for using this type of characteristic should be presented at the fifty-eighth session of the TWV.

PROPOSAL

4. In the previous defined framework, the working group, led by NL and FR, studied the following table showing the *similarities (in italic underlined)* and **differences (in bold)** between the Quantitative (QN) characteristic definition and the proposed new characteristic definition, provisionally named Pseudo Quantitative (PQN) characteristic.

Quantitative characteristics: *QN*	**Pseudo**-*Quantitative Characteristics*: **P***QN*
are those where the expression covers the **full range of variation from one extreme to the other.**	are those which are *quantitative,* where the expression ***does not*** **cover the full range of variation.**
The expression can be recorded on a *one-dimensional, continuous* or discrete, **linear scale.**	The expression can be recorded on a *one-dimensional, continuous* **but nonlinear scale.**
The *range of expressions is divided* into a **number of states** *for the purpose of description* (e.g. length of stem: very short (1), short (3), medium (5), long (7), very long (9)[1]. The division seeks to provide, as far as is practical, an **even distribution across the scale.**	The *range of expressions is divided* in **2 states** *for the purpose of description*, with notes 1 and 2, (e.g. Resistance to ….: **absent or low** (1), **medium or high** (2)). The division provides an **uneven distribution across the scale.**
The Test Guidelines do not specify the difference needed for distinctness[2].	*The states of expression provide distinctness with only 1 note difference, in the case of a condensed scale.*
The states of expression **should**, *however, be meaningful for DUS assessment.*	*The states of expression* ***are****, however, meaningful for DUS assessment.*
[1, 2] Situation validated, but not yet updated in the definition of a Quantitative char.	
[1] *or with a condensed scale: absent or low (1), medium (2), high (3)*	
[2] *The states of expression provide a sufficient distinctness with only 1 note difference, in the case of a condense scale.*	

5. The QN and PQN characteristics are both potentially caused by different combinations of genes (from one gene to gene pyramiding) whose expression could also be influenced by genetic background.

6. In the case of a PQN characteristic, the high resistance level is not identified yet or cannot be distinguished from the level medium resistant, regardless of the test circumstances. Thus, only two reliable notes are proposed.

7. The PQN characteristic could, after a case-by-case study, replace the currently applied QL format (with its two levels of expression). The scale 1- absent or low / 2- medium or high would advantageously replace the scale 1- absent / 9- present, to avoid confusion with the scale dedicated to QL characteristic.

8. To illustrate properly all the states of expression, the working group proposed a panel of examples(See Annex I).

9. The distinctness in the framework of a QN or a PQN characteristic is based on the relevant choice of the threshold varieties which illustrate the cut-off point between 2 UPOV notes by their phenotype.

10. Threshold varieties are special control varieties and must be used to explain the characteristic and define the cut-off points between states of expression. They have to be included in the test. The way to identify and validate a threshold control is detailed in Annex II.

11. Previously adopted type of expression of disease resistance characteristics may need to be revised to confirm their type of expression, scale of notes and explanations on assessment. The TWV may wish to consider commissioning an inventory of disease resistance characteristics in Test Guidelines for future revision.

12. In a preliminary test phase, FR Examination Office and breeders' organizations have begun (March 2024) answering a survey covering all Test Guidelines regardless of the combination Species/Resistance. It has been fruitful to start comparing the proposal of the representatives of the applicants and the FR examination office proposal, to consider the genetic background and to identify some potential revisions of the type of the expression of characteristics.

13. These criteria should be applied to all future Test Guidelines proposals. Experts currently developing or revising Test Guidelines should consider applying these criteria as far as possible. Discussions on applying these criteria to individual Test Guidelines could be organized before the TWV, as required.

14. The TWV is invited to consider possible definition of a new type of expression in UPOV Test Guidelines (or alternatives), with a new note scale, as set out in paragraph 4 of this document.

[Annex I follows]

ANNEX I

Proposed examples to illustrate the used scales

To illustrate the QL scale _____

Example: "Resistance to disease 'x'" with states of expression "absent", note 1; "present" note 9.

e.g. - *Tomato mosaic virus* strain 0 (ToMV:0) in Tomato (TG/44/12(PROJ.4, char.59) - *not yet adopted by the Technical Committee*.

Ad. 59: Resistance to *Tomato mosaic virus* - Strain 0 (ToMV: 0)

[…]

9.3	Control varieties	
	Susceptible	Marmande, Monalbo, Moneymaker
	Resistant to ToMV: 0 and 2	Mobaci
	Resistant to ToMV: 0 and 1	Moperou
	Resistant to ToMV: 0, 1 and 2	"Monalbo x Momor" (with necrosis), Gourmet, Mocimor, Momor

[…]

11.2	Observation scale	symptoms of susceptibility: mosaic in top, leaf malformation symptoms of resistance (based on hypersensitivity): local necrosis, top necrosis, systemic necrosis
11.3	Validation of test	Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls Remark: in some heterozygous varieties a variable proportion of plants may have severe systemic necrosis or some necrotic spots while the other plants have no symptoms. This proportion may vary between experiments.
12.	Interpretation of data in terms of UPOV characteristic states	absent [1] symptoms of susceptibility present [9] no symptoms, or symptoms of hypersensitive resistance

e.g. - *Tomato spotted wilt virus* (TSWV) in Pepper (TG/76/9(PROJ.6, char.62) - *not yet adopted by the Technical Committee*.

Ad. 62: Resistance to *Tomato spotted wilt virus* Pathotype 0 (TSWV: 0)

[…]

9.3	Control varieties	Lamuyo, Yolo Wonder (susceptible), Galileo, Jackal, Jackpot, Prior (resistant)

[…]

11.2	Observation scale	
		Susceptibility: mosaic on young leaf, some leaf malformation
		Resistance: necrosis or only mechanical damage
11.3	Validation of test	Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.
11.4	Off-types	maximum 1 on 20 plants
12.	Interpretation of data in terms of UPOV characteristic states	absent [1] susceptible, see 11.2 present [9] resistant, see 11.2

e.g. - *Passalora fulva* (Pf) in Tomato (TG/44/12(PROJ.4, char.51) - *not yet adopted by the Technical Committee*.

Ad. 51: Resistance to *Passalora fulva* (Pf) - Race 0

[…]

9.3	Control varieties	
	Susceptible	Motelle, Moneymaker
	Resistant	Momor, "Momor x Motelle"
	Remark	"Momor x Motelle" has slightly weaker resistance than Momor

[…]

11.2	Observation scale	Symptoms: Plant death Growth retardation caused by root degradation Root degradation Necrotic pinpoints and necrotic lesions on stems
11.3	Validation of test	Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
11.4	Off-types	
12.	Interpretation of data in terms of UPOV characteristic states	absent [1] symptoms present [9] no symptoms

To illustrate the QN scale

Example: "Resistance to disease 'x'" with states of expression "absent or low", note 1; "medium", note 2; and "high", note 3.

e.g. - *Meloidogyne incognita* (Mi) in Tomato (TG/44/12(PROJ.4, char45) - *not yet adopted by the Technical Committee*.

Ad. 45: Resistance to *Meloidogyne incognita* (Mi)

[…]

9.3	Control varieties	ISF definitions: [4]
	Susceptible	Casaque Rouge
	Intermediate resistant (IR)	Campeon and Tyonic
	Highly resistant (HR)	Arletta, Anahu, Anahu x Casaque Rouge

[…]

11.2	Observation scale	

Class 0: healthy plant, no galls	Class 1: few and little galls which are difficult to find (for example less than 5)	Class 2: few galls, easy to observe but on few roots, still a lot of roots without galls	Class 3: many individual galls on most but not all roots	Class 4: many galls on all roots, sometimes in chains, can lead to dead plants and /or may suppress emergence
				

The germination percentage of non-inoculated plants of the same seed lot in the same experiment should be used to calculate the number of seeds that did not produce a plant due to the presence of nematodes, and add these to plants in class 4.

11.3	Validation of test	Validation on controls. Expected reactions of controls: Susceptible control: - most plants at classes 3 and 4, - at most 2 plants can be observed at class 2 Intermediate resistant control: - clearly different from other controls, - with majority of plants around class 2. Highly resistant control: - most plants at classes 0 and 1, - at most 2 plants can be observed at class 2
11.4	Off-types	Highly resistant varieties may have a few plants with a few galls

12.	Interpretation of data in terms of UPOV characteristic states	Resistance to *Meloidogyne incognita* (Mi): [1] absent or low: distribution of plants in the classes comparable with the susceptible controls. [2] medium: distribution of plants in the classes comparable with the intermediate resistant controls. [3] high: distribution of plants in the classes comparable with the highly resistant controls. If results are not clear, statistical analysis is advised.

e.g. - *Cucumber mosaic virus* (CMV) in Cucumber (TG/61/7 Rev. 2 Corr. 2, char.45)

Ad. 45: Resistance to *Cucumber mosaic virus* (CMV)

[…]

9.3	Control varieties	Bosporus, Corona, Ventura (susceptible) Capra, Gardon, Verdon (moderately resistant) Naf, Picolino (highly resistant)

[…]

11.2	Observation scale	
	[1] susceptible: 3, Corona, Ventura	mosaic; clear border between yellow and green
	[1] susceptible: 4, Bosporus	heavy mottle; confluent chlorosis
	[2] moderately resistant: 5, Gardon, Verdon	light mottle; chlorotic islands
	[2] moderately resistant: 6, Capra	some chlorotic stippling
	[3] highly resistant: 7, Naf, Picolino	no symptoms
11.3	Validation of test	standards should conform to description; describe if different variation within standard should not exceed 1 scale point
11.4	Off-types	2 scale points difference with majority type, maximum 1 out of 6-35 plants
12.	Interpretation of data in terms of UPOV characteristic states	QN [1] 3-4 susceptible, [2] 5-6 moderately resistant, [3] 7 highly resistant

PQN (*which are today identified under the type of expression QL*) _____

Example: "Resistance to disease 'x'" with states of expression "absent or low", note 1; "medium or high", note 2.

e.g. - *Cucumber green mottle mosaic virus* (CGMMV) in Cucumber (2024 partial revision (TWV/58/6, char. 52)

Ad. 52: Resistance to *Cucumber green mottle mosaic virus* (CGMMV)

[…]

9.3¤	Control·varieties¤	Resistance·absent:·Topspin¶ Resistance·present:·Bonaire·(minimum·resistance·level)¶ Bluesbrother·has·higher·resistance·than·Bonaire·¤

[…]

11.2	Observation scale	1) No virus symptoms 2) Isolated yellow spots 3) Mild, localized lesions 4) Wide distribution of mosaic and mottling 5) Strong mosaic, yellowing and distortion of leaf shape

1: no symptoms

3: mild, localized lesions

5: strong mosaic, yellowing and distortion of leaf shape

11.3	Validation of test	On three controls: Topspin, Bonaire, Bluesbrothers, The presence of Bluesbrother (and not only Bonaire) is necessary to validate the aggressiveness of the test.
12.	Interpretation of data in terms of UPOV characteristic states	[1] Resistance absent: comparable with Topspin [9] Resistance present: comparable with Bonaire and Bluesbrother A variety with a lower level of resistance than Bonaire (note 9), will be described as note 1.

e.g. - *Fusarium oxysporum melonis* Race 2 (Fom: 2) in Melon, (TC/59/20, char. 69.3) - *not yet adopted by the Technical Committee.*

Ads. 69: 69.1 - 69.3: Resistance to *Fusarium oxysporum* f. sp. *melonis*, races 0, 1 and 2 (Fom: 0, Fom: 1, Fom: 2)

[…]

9.3.3	Control varieties race 2	Resistance absent: Marianna Resistance present: Perlita, Charentais Fom-1, Védrantais

[…]

11.2	Observation scale	

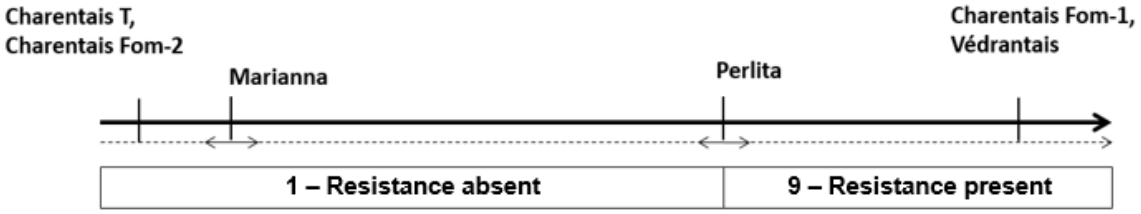
non-inoculated plant	Class 0	Class 1
At least 5 plants	Healthy plant: no symptoms of yellowing and wilting, could be some growth reduction due to inoculation stress compared to mock. Sometimes in the mock we can observe some yellowing, different from the symptoms of *Fusarium*	Light symptoms of yellowing/wilting
		

Class 2	Class 3	
typical symptoms: yellowing, wilting and necrosis, stunting (growth stopped)	Death of plant (Dead)	
		 Other symptoms of vein clearing could be difficult to judge. It is advised to make a later notation to observe the evolution of these symptoms over the time.

Courtesy of GEVES-SNES in the framework of CPVO Harmores project.

11.3	Validation of test	In case of the Fom: 2 test Controls expected response: • Susceptible controls, with UPOV characteristic state 'Resistance absent', should have most of the plants in observation classes 2 or 3, and few or no plants in observation classes 0 or 1. ○ Marianna, the susceptible control is less susceptible than Charentais Fom-2, Charentais T • Resistant controls should have most of the plants in observation classes 0 or 1, and few or no plant in observation classes 2 or 3. Perlita, the lower threshold resistance control, should have at least some plants in observation class 1, 2, or 3. It has to be less resistant than Charentais Fom-1, Védrantais.

11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	In case of varieties with a response between the susceptible (resistance absent) and the resistant control, repeat the test. In case of confirmation of the result, the variety will be judged heterogeneous. In case of unclear results, retest or test in another lab.



e.g. - *Fusarium oxysporum f.sp lycopersici* (Fol) , race 0 and 1, in Tomato (TG/44/12(PROJ.4, char. 83) - *not yet adopted by the Technical Committee*.

Ad. 47: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* - Race 0EU/1US (Fol: 0EU/1US)

[…]

9.3	Control varieties	
9.3.1	Control varieties for the test with race 0EU/1US	Susceptible: Marmande, Marmande verte, Resal, Moneymaker Resistant: Marporum, Larissa, "Marporum x Marmande verte", Motelle, Gourmet; and Riesling as additional resistant control for medium level
9.3.2	Control varieties for the test with race 1EU/2US	Susceptible: Marmande verte, Cherry Belle, Roma, Marporum, Ranco, Moneymaker Resistant: Tradiro, Motelle, "Motelle x Marmande verte"; and Agostino as additional resistant control for medium level

[…]

11.2	Observation scale	

Class 0	Class 1	Class 2	Class 3
Healthy compared to the non-inoculated control.	Healthy compared to the non-inoculated control with brown vessel above the cotyledon (observed when plants are cut in case of variety with different levels of symptoms)	Higher than 50% of growth reduction and/or yellowing and/or wilting on cotyledons and/or leaves.	Nearly dead: strong reduction with plants look dwarf (there can be necrosis but not always) or dead
			

If all plants in class 0 or if all plants in classes 2 and 3, it is not necessary to cut the plants.

In case of variety or control with different levels of symptoms, cut the plants to check presence or not of strong brown vessel above cotyledons.
In case of no brown vessels or below cotyledons, the plant is note 0. In case of brown vessels above cotyledons, the plant is note 1.

11.3	Validation of test	Validation on controls. Expected response of controls: Susceptible control: most plants in class 2 and 3, max.10% of plants class 0 and 1 Resistant control: most plants in class 0 and 1, max. 10% of plants class 2 and 3. Controls with medium level of resistance can show a higher number of plants in class 2 and 3.
12.	Interpretation of data in terms of UPOV characteristic states	[1] absent: Average symptom level higher than in the medium-resistant control [9] present: Average symptom level not different from the medium-resistant control or the high-resistant control If no clear results, statistics may be used.

In some cases, information may not be available for certain levels of susceptibility / resistance, such as when there are no known varieties with high level of resistance to illustrate a particular QN characteristic.

e.g. - *Watermelon mosaic virus* (WMV) in Squash (TC/59/24, char. 83*) - not yet validated by TC-EDC*.

Ad. 83: Resistance to *Watermelon mosaic virus* (WMV)

[…]

9.3	Control varieties	To illustrate UPOV notes: • resistance absent: Cora • resistance present: Sofia (minimum resistance level) Mikonos, Syros have higher resistance than Sofia, but not resistant enough to illustrate a high resistance.

[…]

11.2	Observation scale	Class 0: no symptom Class 1: few small chlorotic patches Class 2: many chlorotic patches Class 3: large chlorotic areas (some patches on young leaves) Class 4: mosaic, weak vein banding Class 5: deformation and vein banding

0: no symptom

1: few small chlorotic patches

2: many chlorotic patches

3: large chlorotic areas (some patches on young leaves)

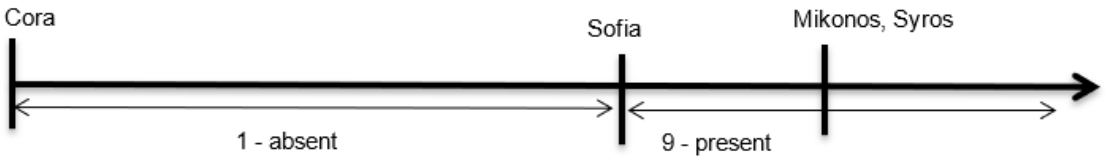
4: mosaic, weak vein banding

5: deformation and vein banding

Courtesy of GEVES-SNES

11.3	Validation of test	On three controls: Cora, Sofia, Mikonos or Syros The presence of Syros or Mikonos (and not only Sofia) is necessary to validate the aggressiveness of the test. Results should be compared with the results of controls, based on disease index (DI) and distribution of plants over the classes.
12.	Interpretation of data in terms of UPOV characteristic states	Note 1: Most plants are in class 4 and/or 5 (resistance absent or low to be considered) Note 9: Most plants are in class 0, 1, 2 and/or 3 (resistance present -more or less intensely) A variety with a lower level of resistance than Sofia (note 9), will be described as note 1 An additional statistical analysis can be used to finalize the pathologist's raw observation to the assessment of uniformity, and relative position regarding the controls results.

Resistance to WMV:



[Annex II follows]

ANNEX II

How to identify and validate a threshold control?

Several steps have to be fulfilled before the validation of a threshold variety, such as:

- To identify the threshold control candidates amongst varieties with well identified resistance genetic and / or correlated with field observations.
- To be included in a panel of potential controls, in an international R&D project, involving interested examination offices regularly performing the test for DUS purpose, and disease resistance labs of applicants
- To regularly show the expected behavior, in the framework of a finalized and published disease resistance test protocol.
- To be maintained and available from by reliable initiatives (e.g: MATREF (FR), PLANTUM (NL), CPPSI (USA)…), identified in an international database such as HARMORESCOLL – EU
- The behavior of a threshold variety does not have an absolute value by itself, but it obtains a strategic value in a validated test, including the other required controls, to allow the validation of the test (aggressiveness, number of tested plants to allow potential statistical assistance, required season if required….)

[End of Annex II and of document]