UPOV
International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables

TWV/53/13 Rev.

Fifty-Third Session
Seoul, Republic of Korea, May 20 to 24, 2019

Original: English
Date: May 29, 2019

USE OF DISEASE RESISTANCE CHARACTERISTICS

Document prepared by the Office of the Union

Disclaimer: this document does not represent UPOV policies or guidance

1. The Technical Working Party for Vegetables (TWV), at its fifty-second session held in Beijing, China, from September 17 to 21, 2018, agreed that, looking at the increase of the use of disease resistance characteristics in DUS examination for vegetables, it would be useful to add a new agenda item in that respect. In particular, it proposed to invite presentations from France, the Netherlands, ISF and any other members and observers on the topic of standardization of the methodology, to understand better the different approaches used by pathologists, breeders and DUS examiners (see document TWV/52/20 "Report", paragraph 51).

2. This document contains presentations made at the fifty-third session of the Technical Working Party for Vegetables (TWV):

Annex I "Use of disease resistance characteristics", presented by an expert from the European Union
Annex II "Evaluation of disease resistance in vegetable varieties according to UPOV standards. A focus on the Italian activities", presented by an expert from Italy.
Annex III "Disease resistance in DUS", presented by experts from France and the Netherlands.
Annex IV "Harmonization of resistance tests to diseases for DUS testing: Harmores 3", presented by an expert from France
Annex V "Disease resistance in vegetables: What does the European industry do in terms of claims?", presented by an expert from the European Seed Association (ESA).
Annex VI "ISF Working Group Disease Resistance Terminology", presented by an expert from the International Seed Federation (ISF).

[Annexes follow]

CPVO
Community Plant Variety Office

Use of Disease resistance characteristics

TWV/53 – Seoul, South Korea, 2019
Céline Morineau

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Community plant variety office

- We grant Intellectual Property Rights for plant varieties valid throughout the European Union
- We deal with applications (reception, organisation and monitoring of the technical examinations)
- We do not perform the DUS testing

-> collaboration with the Examination offices in EU

- Our CPVO Technical protocols are developed on the basis of the UPOV Test Guidelines

Basis of the approach from CPVO

- the resistance characteristics we introduce in our TP should match the UPOV requirements for a characteristic:

- sufficiently consistent and repeatable in a particular environment
- exhibits sufficient variation between varieties to be able to establish distinctness
- capable of precise definition and recognition
- allows uniformity and stability requirements to be fulfilled

Basis of the approach from CPVO

Types of expression for resistance characteristics: QL and QN

Definitions according to UPOV TG/1/3

4.4.1 Qualitative Characteristics
…are expressed in discontinuous states. These states are self-explanatory and independently meaningful. All states are necessary to describe the full range of the characteristic, and every form of expression can be described by a single state. The order of states is not important. As a rule, the characteristics are not influenced by environment.

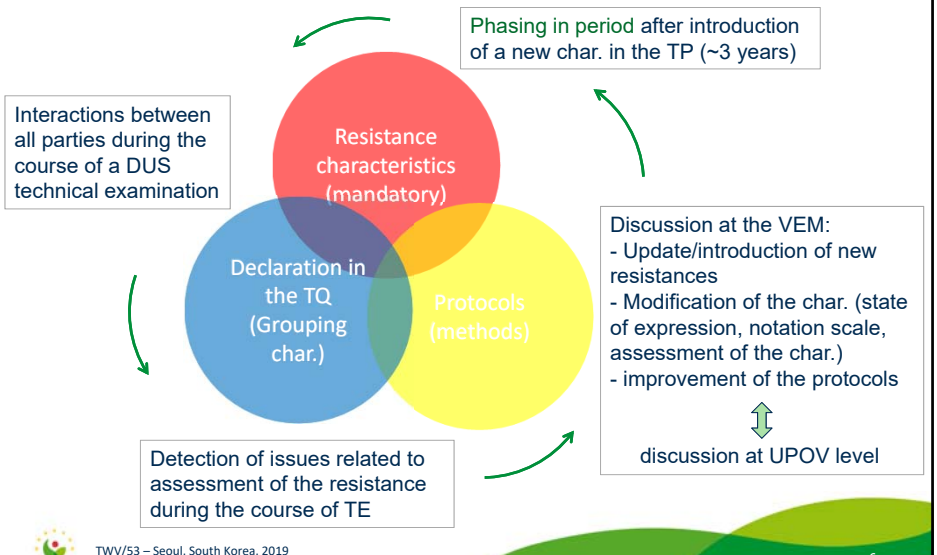
4.4.2 Quantitative Characteristics
…the expression covers the full range of variation from one extreme to the other. The expression can be recorded on a one-dimensional, continuous or discrete, linear scale. The range of expression is divided into a number of states for the purpose of description. The division seeks to provide, as far as is practical, an even distribution across the scale. The Test Guidelines do not specify the difference needed for distinctness. The states of expression should, however, be meaningful for DUS assessment.

Basis of the approach from CPVO

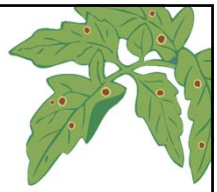
- Development of the protocols to test resistance based on existing literature
- Occasional support to more up-stream research (R&D projects coordinated by research institutes)

- UPOV model 1: Markers replace the biotest -> direct correlation between the phenotype and the marker (100% of reliability)
- Biotest + MM: molecular markers as a support of the decision and give help to interpretation

Close collaboration between Examination offices and breeders at all steps



CPVO R&D projects related to the use of the resistance characteristics



The CPVO promotes the harmonisation of the methods

→ common interpretation of symptoms between EOs

- support of Harmores projects I, II and III leading to the harmonisation in EU of
  - resistance protocols
  - references varieties
  - isolates, differentials
- It concerns 15 diseases
- Total budget ~ 506 000 euros

Overview of CPVO TPs including resistance characteristics

12 CPVO TPs
(55 CPVO TPs in total for vegetables)

↓

Up to 55 diseases
(crop species / pathogen species)
55 methods described in the TP for the testing of the resistances

↓

Up to 109 resistance characteristics
(~ crop species / pathogen race couples) are included in the CPVO TPs

Overview of CPVO TPs including resistance characteristics

COMMON NAME	NB RESISTANCE CHAR.	NB DISEASES	ALL CHAR. ARE QL (1-9) EXCEPT FOR THE FOLLOWINGS THAT ARE QN (1-2-3)	TWO DISEASES CAN BE TESTED BY MARKERS AS REPLACEMENT OF METHOD OF BIOTEST
lettuce	18	4	*Fusarium oxysporum* f.sp. *lactucae* (Fol) race 1	
tomato	25	16	*Meloidogyne incognita* (Mi)	- *Tomato mosaic virus* (ToMV), strain 0, 1 and 2 - *Tomato spotted wilt virus* (TSWV) - Strain 0
tomato rootstocks	20	11	same as for tomato	same as for tomato
pea	5	3		
french bean	5	4		
cabbage	1	1		
spinach	16	2		
cucumber, gherkin	8	8	- *Cucumber mosaic virus* (CMV) - powdery mildew (*Podospaera xanthii*) (Px) - downy mildew (*Pseudoperonospora cubensis*) (Pc)	
cornsalad	2	1		
melon	14	8	- *Sphaerotheca fuliginea* (*Podosphaera xanthii*) (Powdery mildew), Race 1, 2 and 5 - *Erysiphe cichoracearum* (*Golovinomyces cichoracearum*) Race 1 (Powdery mildew) (*Fusarium oxysporum* f. sp. *melonis*, Race 1-2)	
pepper	11	6		
watermelon	4	2		
Total	109	55		

7 resistance characteristics are QN among 109

2 diseases (~ 4 resistance char.) can be assessed by MM

Overview of CPVO TPs including resistance characteristics

COMMON NAME	NB RESISTANCE CHAR.	NB DISEASES	ALL CHAR. ARE QL (1-9) EXCEPT FOR THE FOLLOWINGS THAT ARE QN (1-2-3)	TWO DISEASES CAN BE TESTED BY MARKERS AS REPLACEMENT OF METHOD OF BIOTEST	HARM. I, II, III
lettuce	18	4	*Fusarium oxysporum* f.sp. *lactucae* (Fol) race 1	-	1
tomato	25	16	*Meloidogyne incognita* (Mi)	- *Tomato mosaic virus* (ToMV), strain 0, 1 and 2 - *Tomato spotted wilt virus* (TSWV) - Strain 0	4
tomato rootstocks	20	11	same as for tomato	same as for tomato	4
pea	5	3	-	-	3
french bean	5	4	-	-	3
cabbage	1	1			
spinach	16	2			
cucumber, gherkin	8	8	- *Cucumber mosaic virus* (CMV) - powdery mildew (*Podospaera xanthii*) (Px) - downy mildew (*Pseudoperonospora cubensis*) (Pc)		
cornsalad	2	1			
melon	14	8	- *Sphaerotheca fuliginea* (*Podosphaera xanthii*) (Powdery mildew), Race 1, 2 and 5 - *Erysiphe cichoracearum* (*Golovinomyces cichoracearum*) Race 1 (Powdery mildew) (*Fusarium oxysporum* f. sp. *melonis*, Race 1-2)	-	2
pepper	11	6	-	-	2
watermelon	4	2			
Total	109	55			15

[Annex II follows]

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Technical Working Party for Vegetables UPOV-TWV
53rd Session

Evaluation of disease resistance in vegetable varieties according to UPOV standards. A focus on the Italian activities

Romana Bravi
CREA-Research Centre for Plant Protection and Certification
Bologna- Italy

Loredana Sigillo
CREA-Research Centre for Vegetable and Ornamental Crops
Pontecagnano (SA)-Italy

20-25/05/2019, Seoul – Republic of Korea

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Outline

1. Disease resistance characteristics –UPOV Test Guidelines
2. UPOV requirements
3. Strengths and weaknesses of use
4. A focus on Italian activities (CREA)
5. Disease resistance protocols applied
6. Main critical issues
7. Future perspective

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Disease resistance characteristics - UPOV

- **Physiological characteristics (TGP/12)**

- **Characteristics based on a response to an external factor as living organism (pathogens and pests)**
- **Use in Registration/Protection for DUS test and breeding/genetic resources**
- **Diseases resistance characteristiscs in UPOV vegetables and agricultural crops Test Guidelines**
- **Increasing use (sustainable agriculture)**
- **Possibility to make disease resistance characteristic compulsory (*)**

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Disease resistance characteristics – UPOV Test Guidelines

Since 1970's : tomato (*TMV, Fusarium, Verticillium, Nematodes*); **bean** (*Collethotricum, BCMV);* **pea** (*Fusarium pisi, Ascochita pisi*)

VEGETABLES : *tomato(21+4*), tomato rootstock (14 +6*), pea (5), French bean (3 +2 *), lettuce (16), melon (13+3*), cucumber (7), pepper (8+4*), spinach (16),cornsalad (2)* , cabbage(1), watermelon (4)

AGRICUTURAL CROPS : *lucerne* - VCU traits
Ornamental and fruit crops: no one (not necessary for D)

Additional sources of variation :

- ❑ the effects of factors (light, temperature, humidity) on the development or aggressivity of the pathogens
- ❑ genetic variability of the pathogens

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UPOV requirements

- **UPOV requirements are that characteristics should fulfill (TG/1/3)**
- **Appropriate methods under controlled conditions and recognised standards and protocols (bioassay)**

Expression of disease resistence characteristic should fulfill :

- Knowledge of nature of genetic control
- Consistent and repeatable (standardization of conditions of fields, greenhouse, laboratory , appropriate protocols, validated methodology)
- Sufficient variation between varieties, clear differentiation
- Precise definition and recognition (type of response and state of expressions) QL,PQ,QN
- Uniformity and stability requirements

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Use of disease resistance characteristics

Strengths

Advantage for Examination Authorities :

1. **Greater possibilities for distinctness (high discriminatory power: usually QL)**
2. **Assists the management of variety collection**
3. **Optimize set-up of DUS trial (grouping of varieties)**
4. **Use of molecular test**

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Use of disease resistance characteristics

Weaknesses

✓ **Limit in the use, pratical difficulties for Examination Authorities:**

1. **Investment required: financial, facilities, wide expertise**
2. **Reliable methodologies, technical requirements (constant and repeatable results)**
3. **Requirements of uniformity**
4. **Periodic review of protocol**
5. **Quarantine regulation**
6. **Availability reliable inoculum**
7. **Interaction with genetic background (new genes of resistences)**
8. **Outsourcing of testing**

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A focus on diseases resistance testing for DUS – CREA (Research Centre fo Plant Protection and Certification)

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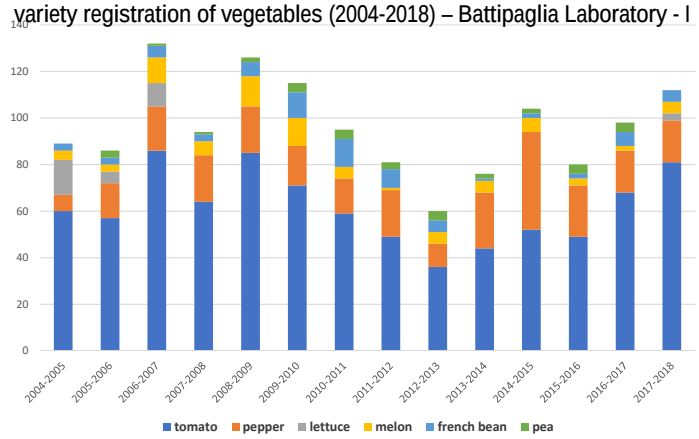
Experience in Italian laboratories (CREA) in the framework of variety registration

Number of samples tested for disease resistance characters in the framework of variety registration of vegetables (2004-2018) – Battipaglia Laboratory - I



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Disease resistance protocols applied in routine for registration

Species	Pathogen	remarks
Tomato	*Meloidogyne incognita*	
	Verticillium dahliae	
	Fusarium oxysporum f. sp. *lycopersici* race 0 (ex 1) and 1 (ex 2)	
	Tomato mosaic virus race 0, 1, 2 and 1-2	
	Tomato spotted wilt virus race 0	Non-compulsory
	Tomato yellow leaf curl virus	Non-compulsory, external laboratories **INIA ; CNR (IT)**
	Phytophthora infestans	Non compulsory
	Cladosporium fulvum race 0	Non-compulsory
Pepper	Potato virus y pathotype 0	
	Tobamovirus race 0; race1-2; race 1-2-3	
	Tomato spotted wilt virus race 0	Non-compulsory
Melon	*Fusarium oxysporum* f. sp. *melonis* race 0, 1, 2 and 1,2	
French bean	*Colletotrichum lindemutianum* race lambda and 6	
	Baen common mosaic virus strain NL5	
Pea	*Fusarium oxysporum* f. sp. *pisi* race 1	
	Erysiphe pisi	Non-compulsory
Lettuce	Lettuce mosaic virus strain Ls1	Non-compulsory
	Bremia lactucae races	External laboratories **: GEVES, Naktuinbouw**

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Scope, validation and critical points – TOMATO-resistance protocols

Scope	Pathogen	Validation	Critical points
	Meloidogyne incognita	✓ Internal validation	Mantainanche of alive inoculum
	Verticillium dahliae	✓ Internal validation	Need of a common reference isolate / segregation of the character
	Fusarium oxysporum f. sp. *lycopersici* race 0 (ex 1) and 1 (ex 2)	✓ Validation during Harmores 3 project	Validation of lab. reference varieties / interpretation of IR characters
	Tomato mosaic virus race 0, 1, 2 and 1-2	✓ Internal validation	New validation for race 1, 2 and 1-2 if needed
	Tomato spotted wilt virus race 0	✓ Internal validation	Loss of virulence of the strains, quarantine status of the pathogen
	Tomato yellow leaf curl virus	✗ Not validated	Tests are carried out by external accredited laboratories (INIA,ES -CNR, IT) / quarantine status of the pathogen
	Phythophtora infestans	✗ Not validated	Unsuccesfull set up (loss of virulence of the strain). Interference of Botrytis
	Cladosporium fulvum	✓ Internal validation	Inoculation method / pathogenicity of the strains / identification of races
	Pseudomonas syringae pv *tomato*	✓ Internal validation	Need of harmonized protocol and reference materials
	Pyrenochaeta lycopersici	Validation in progress	Need of harmonized protocol and reference materials
	Ralstonia solanacearum	✓ Internal validation	Need of reference material / Quarantine status of the pathogen

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Segregation of the characteristic of resistance to *Verticillium dahliae* in tomato varieties

The expression of character is evaluated heterogeneous when the number of off-types exceed the maximum standard allowed (1/20). The bioassay is repeated by the EO in case of lack of uniformity. If the result is confirmed, the characteristic is considered «**segregant**».

Cycle	Segregant varieties for resistance *to V. dahliae* (%)
Spring 2016	20,03
Spring 2017	17,86
Spring 2018	11,63

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Segregation of the character of resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 1 in tomato varieties

The expression of character is evaluated heterogeneous when the number of off-types exceed the maximum standard allowed (1/20). The bioassay is repeated by the EO in case of lack of uniformity. If the result is confirmed, the characteristic is considered «segregant».
Some samples have a ***continuum*** variation of the expression of the resistance. Are they intermediate resistant (IR)? Waiting for the results of Harmores 3 project to give an harmonized answer.

Heterogeneous

Level 0,1, 2
Intermediate resistant?
To be compared with an IR reference varieties

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Resistance protocols
Scope, validation and critical points

MELON

Scope	Pathogen	Validation	Critical point
	Fusarium oxysporum f. sp. *melonis* race 0, 1 and 2 ✓	During Harmores 3 project	
	Fusarium oxysporum f. sp. *melonis* race 1,2 ✓	During Harmores 3 project	Intermediate resistance

FRENCH BEAN

Scope	Pathogen	Validation	Critical point
	Colletotrichum lindemuthianum race lambda and 6 ✓	Internal validation	
	Bean Common Mosiac Necrotic Virus strain NL5 ✓	Internal validation	Test validated only in glass-house
	Xanthomonas axonopodis pv. *phaseoli* ⚙	Internal validation in progress	Quarantine pathogen / validation with an Italian isolate

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Resistance protocols
Scope, validation and critical points

PEPPER

Scope	Pathogen	Validation	Critical points
	Potato virus Y pathotype 0	Internal validation (according Harmores 2 results)	Availability of reference varieties
	Tobamovirus race 0, 1-2 and 1-2-3	Internal validation (according Harmores 2 results)	Availability of reference varieties / contamination of strains
	Tomato spotted wilt virus race 0	Internal validation	Quarantine pathogen / validation with an Italian isolate

PEA

Scope	Pathogen	Validation	Critical points
	Fusarium oxysporum f. sp *pisi* race 1	Internal validation (according Harmores 2 results)	Availability of reference varieties
	Erysiphe pisi	Internal validation	Validation in open fields

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Resistance protocols
Scope, validation and critical points

LETTUCE

Scope	Pathogen	Validation	Critical points
	Bremia lactucae races	Not validated	Analyses carried out by external laboratories : GEVES (FR), Naktuinbouw (NL))
	Lettuce mosaic virus strain Ls1	Validation in progress	
	Fusarium oxysporum f. sp. *lactucae* race 1	Internal validation	

SPINACH

Scope	Pathogen	Validation	Critical points
	Peronospora farinosa f.sp.spinaciae races	Not validated	Analyses carried out by external laboratories (GEVES (FR), Naktuinbouw (NL)

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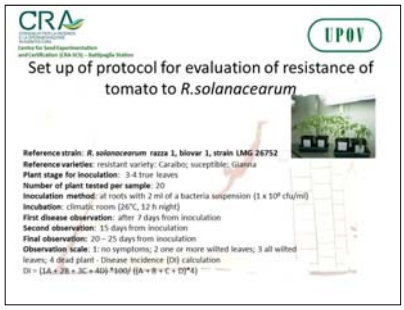
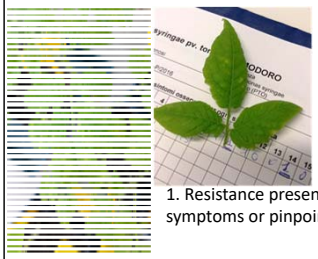
Italian experience in assistance to breeders – TOMATO

Ralstonia solanacearum



Resistance to ***Pseudomonas syringae* pv. *tomato*** - CPVO TP44/4 rev.

Notation scale adopted



1. Resistance present – no symptoms or pinpoint lesions

9. Resistance absent - Bacterial speck

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Italian experience in assistance to breeders - TOMATO rootstock

Set up of the bioassy protocol to evaluate the resistance to *Pyrenochaeta lycopersici* - in progress in 2019

- Choice of reference strain and establishment of pathogencity on susceptible variety ✓
- Choice of reference susceptible and resistant variety ✓
- Establishment of test condition (20 - 23 °C in climatic chamber) ✓
- Preparation of the inoculum (development of the isolate on mile grains) ✓
- Definition of notation scale
- Definition of interpretation rules

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Italian experience in assistance to breeders LETTUCE


XVIII Convegno Nazionale
Società Italiana di Patologia Vegetale
Sassari 24 - 26 Settembre 2012

A STANDARDIZED METHOD TO EVALUATE THE RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *LACTUCAE* IN LETTUCE

Loredana Sigillo[1], Veronica Senape[1], Giovanna Serratore[1], Romana Bravi[1]

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Fusarium oxysporum f. sp. *lactucae* (Fol) is the causal agent of fusarium wilt in lettuce. The widespread of the disease is increasing especially in the fourth range productions. Three races of the pathogen are known but in Italy only race 1 affects cultivations. Traditional control methods are not effective and the use of resistant varieties can reduce yield losses.

Reference strain of *Fusarium oxysporum* f. sp. *lactucae* race 1

The characteristic "resistance to Fol" is becoming more and more important in commercial varieties. However its evaluation is not included in the official description of lettuce varieties for Listing in National and European Catalogues; the listing is based on the examining of the variety Distinctiveness, Uniformity and Stability (DUS test), according to the CPVO technical protocols and UPOV technical guidelines.

A method to evaluate lettuce variety resistance to Fol has been developed on demand of seed companies. The test is based on the dipping root inoculation method and is performed in climatic room conditions.

The observation of the symptoms have to be reported according to a disease scale and the symptoms developing on the samples are compared with those observed in reference varieties.

Observation scale

Symptoms	Disease code	No. of plants
Healthy	0	A
Slightly stunting, growing reduction	1	B
Severely stunting	2	C
Dead	3	D

After statistical elaboration on about 30 varieties, results showed that the variety behaviour varies from resistant to intermediate resistant and susceptible.

Disease incidence (%)	Level of resistance
0 - 10	Resistant
11 - 20	Intermediate resistant
21 - 80	Susceptible
81 - 100	Very susceptible

During the last Technical Working Party for Vegetables (Venlo, NL, June 2012) UPOV specialists from several countries discussed and accepted this protocol to test Fol resistance in lettuce. Although resistance to Fol is a quantitative characteristic, to allow a simplification in DUS testing, UPOV technical protocol deal it as a qualitative one identifying only two level of characters expression (susceptible/resistant) and considering "intermediate resistant", "susceptible" and the "very susceptible" together as "susceptible".

Resistance to *Fusarium oxysporum* f. sp. *lactucae* race 1

- Validation of isolate and reference varieties
- Definition of inoculation method, growing conditions, disease scale and interpretation rules
- A revision of the protocol for new isolates and interpretation rules is planned

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Services to companies for resistance characterstics not included in UPOV/CPVO technical protocols

Bioassy for resistance

Species	Pathogen
Tomato	*Clavibacter michiganensis* subsp. *michiganensis*
Basil (Ocimum basilicum)	*Peronospora* spp.
	Fusarium oxysporum f. sp. *basilici*
Wild rocket (Dyplotaxis tenuifolia)	*Fusarium oxysporum* f. sp. *raphani*
Eggplant rootstock	*Fusarium oxysporum* f. sp. *melongenae*
	Verticillium dahliae
	Pyrenochaeta lycopersici
Chickpea (Cicer aretinum)	*Ascochyta rabiei*

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The main critical issues

- **Laboratory structure (facilities,high-tech equipment) and wide-ranging expertise (the tests have to be carried out in different conditions for different pathogens)**
- **Discontinous activity (low number of samples/species/year, or high number of compulsory tests, increasingly application of non-mandatory tests)**
- **Managing of biotrophic and quarantine pathogens**
- **Availability of some reference varieties and set of differential varieties**
- **Establishment of conditions for a save conservation of pathogen collections**

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Future perspective of diseases resistance testing activities (CREA)

- Concentration of disease resistance testing in Central Laboratory of CREA in Rome
- Specialization only on specific disease resistance tests (Battipaglia laboratory)
- Use of expertise of pathologist of the research Centre
- Outsource disease resistance tests
- Cooperation with other Examination Office
- Cooperation with breeders
- Participation to ring test or research projects

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[Annex III follows]

Disease resistance in DUS

UPOV TWV/53, Seoul

France and The Netherlands

Chrystelle Jouy, GEVES and Amanda van Dijk, Naktuinbouw

Growing vegetables

To spray or not to spray, that's the issue. Very important element in **sustainability**.

Growers prefer resistant varieties. A resistant variety does not suffer (or less) from the disease. The disease is in the air or in the soil, but the production is not substantially affected.

Diseases do not only influence yield, but also visual quality of the product. **Consumers** prefer disease and pesticide free products.

Breeding vegetables

To fulfil the need of the grower new varieties are bred, more and more resistant. Important **innovation**. Decrease of use of chemicals wanted/needed.

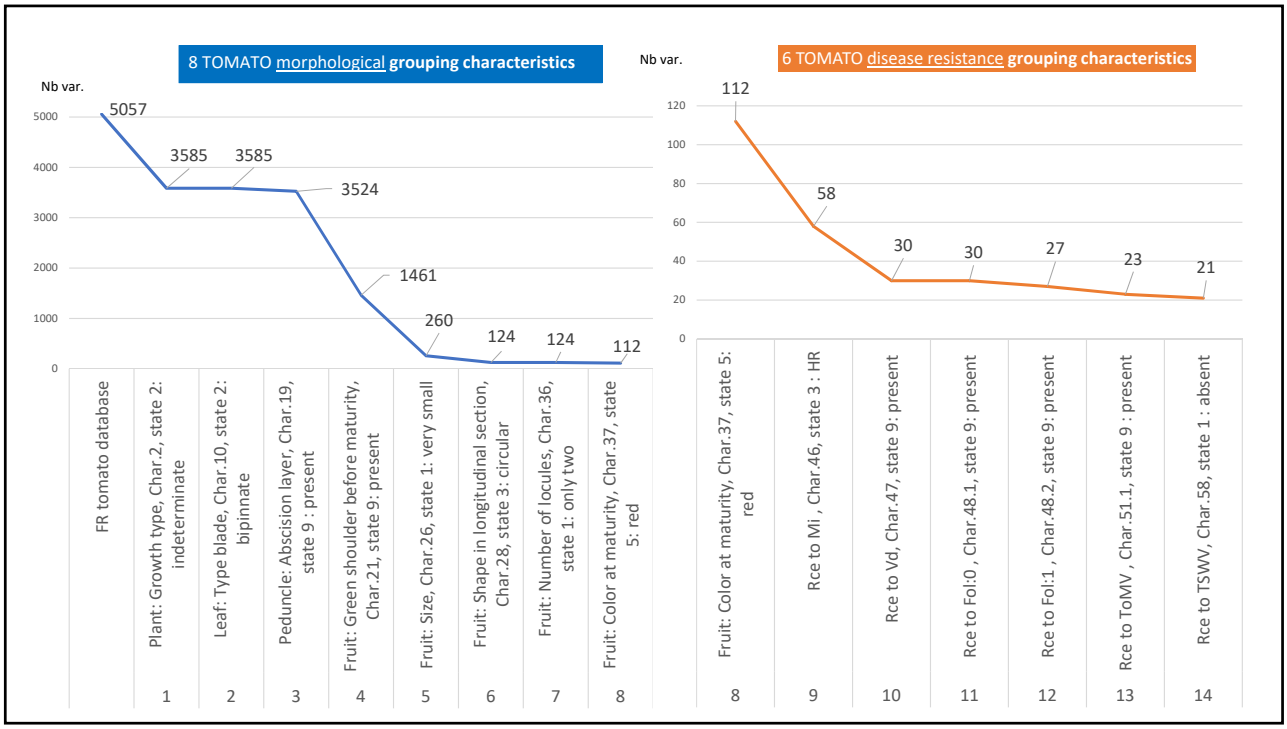
In the breeding process and in the quality control of varieties, resistance is checked routinuously.

Efficient tests have been developed: breeders need a test which is cheap, fast and well correlated to meaningful resistance for the grower.

Innovation needs protection.

DUS tests in vegetables

- Very important, resistance characteristics allow **grouping of varieties**.
- Dividing the variety collection into groups (for example in a susceptible and a resistant group) makes the DUS test **much more efficient**. It decreases largely the number of comparing varieties to be included in the trial.
- Differences in resistance often do not express clear morphological differences. This would mean rejection of varieties with clearly better features on **lack of distinctness**. Fortunately, resistance characteristics fulfill UPOV characteristic criteria and can be used in the DUS decision.



Example of added efficiency in the DUS trial of white cabbage (*Brassica oleracea*) when using Resistance to *Fusarium oxysporum* f.sp. *conglutinans* in grouping varieties.

All varieties	548
Grouped with Foc resistance	233
Grouped with cms	94
Grouped with 12 morphological characteristics	6

Source: NL database white cabbage

Resistance tests in DUS

Each resistance characteristic has an **Explanation**: a test protocol, preferably well correlated to meaningful resistance for the grower. The protocol should be validated, harmonized and efficient, reproducible, robust.

Efficient: generally a test on seedlings. Not always easy: interaction between two living agents (pathogen and plant). Control of test circumstances is critical.

Elements in the Test Guidelines:

- Characteristic with its states of expression, QN, PQ or QL, how many states, example varieties
- Test protocol: test method, control varieties, observation scale and interpretation of data into UPOV characteristic states of expression

Usually…

Breeders use a **strong, dominant gene** which gives a clear result in the efficient test: the seedlings die (variety is susceptible) or the seedlings look quite healthy (variety is resistant).

Some variation will occur from plant to plant or from test to test: the expression of the gene is influenced by the relation between disease and plant, and by test conditions.

Developments…

1. Use of **new genes or QTLs** (from wild material) could lead to lower levels of resistance while for the grower the resistance is still strong enough when disease pressure is not too high. This wider gene pool assists the **durability of the resistances.**
2. New genes or QTLs (*even when not related to the resistance*) **may influence the expression** of the strong, dominant gene.
3. Definition by ISF:

- **High resistance (HR):** plant varieties that highly restrict the growth and/or development of the specified pest and/or the damage it causes under normal pest pressure when compared to susceptible varieties. These plant varieties may, however, exhibit some symptoms or damage under heavy pest pressure.
- **Intermediate resistance (IR):** plant varieties that restrict the growth and/or development of the specified pest and/or the damage it causes but may exhibit a greater range of symptoms or damage compared to high resistant varieties. Intermediate resistant plant varieties will still show less severe symptoms or damage than susceptible plant varieties when grown under similar environmental conditions and/or pest pressure

Also in seedling test different levels can be studied.

4. Fear of damage claims by growers in case of disease in a field makes that seed companies sometimes choose **to use the wording 'intermediate resistant' instead of 'high resistant',** even if it concerns a true high resistance. (isolates breaking resistance, high temperature breaking resistance, ie: TSWV in Tomato or Peppers, *Meloidogyne* in Tomato))

Elements in the UPOV Test Guidelines

To be able to use a resistance characteristic in DUS tests

- The characteristic must be suitable for **uniformity** judgement
- The combination of the **indication QL/QN/PQ** and the **states of expression** should not lead to incorrect decision on distinctness

Currently, many resistance characteristics have the indication QL with state 1 (absent) and 9 (present).

Since a number of years some characteristics have the indication QN with state 1 (susceptible), 2 (moderately resistant) and 3 (highly resistant). Varieties with state 2 are according to UPOV rules not normally distinct from varieties with note 1 or 3, if that is the only difference.

Challenge: IR

- We need **correct decisions** on DUS and a correct **Official Description**
- We need resistance characteristics **in grouping**
- **Breeders use** IR/HR, at present UPOV uses R (sometimes moderately)
- Varieties developped with **new resistances** and **genetic backgrounds**

- The good news: more detailed knowledge on genetics available … for a better assessment of morphological Distinctness.

What to know and to decide before a resistance characteristic is proposed?

1. **Genetics of the resistance** (genotype)
   It is helpful to know the genetics of the resistance. Based on 1 gene or more? Dominant or co-dominant behaviour? Do other genes in the background play a role? Important element to decide on QL/QN/PQ.
2. **Symptoms of the disease** (phenotype)
   Symptoms shown in a continuous or discrete scale? Is the observation scale one-dimensional? Element to decide on QL/QN/PQ. The scale is specific for this test on this disease.
3. **Interpretation of data in terms of UPOV characteristics**
   Data of the observation scale are translated into a reduced number UPOV states as a reproducible combination of genotype and phenotype, useful for distinctness and description, as far as possible related to needs of the grower. Illustrated by example varieties.

QL, PQ or QN characteristic

UPOV definition

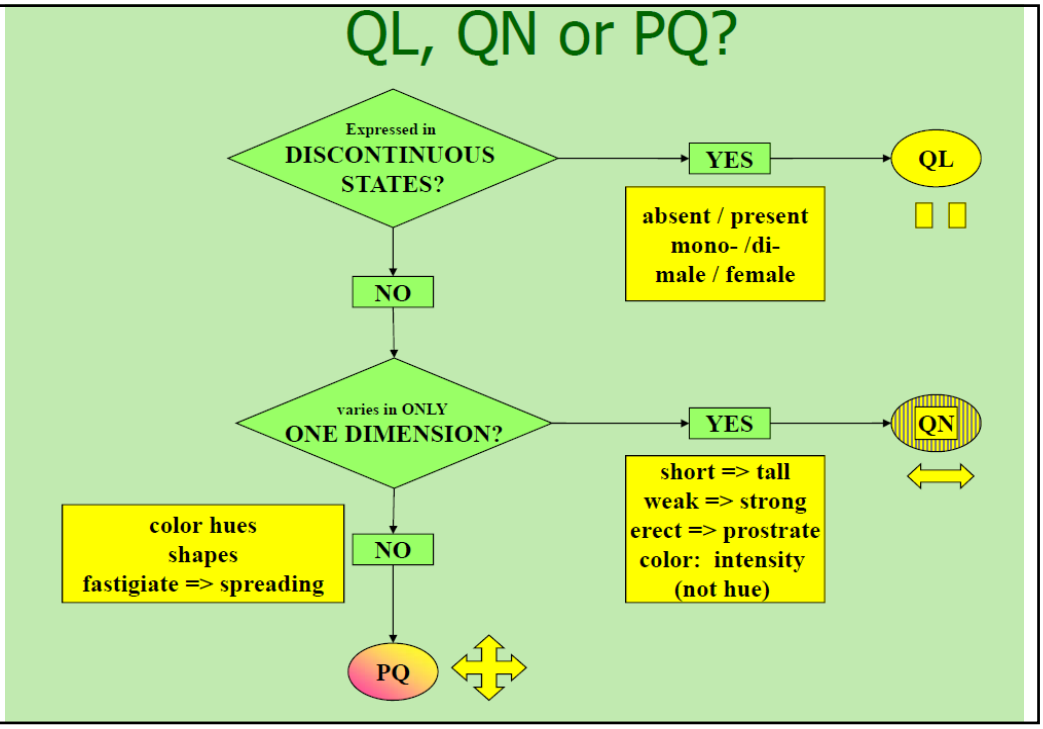
QuaL**i**tative: discontinuous states, states are self-explanatory, states are independently meaningful, order of the states is not important

Pseudo**Q**ualitative: variation in more than one dimension, partly continuous, cannot be adequately described by just defining two ends of a linear range

QuaN**t**itative: expression covers the full range of variation from one extreme to the other, can be recorded on a one-dimensional, continuous or discrete, linear scale, even distribution across the scale.

Example: Plant: length. Polygenetic base, genetic causes several levels. Varieties are distributed all over the scale.

https://www.upov.int/edocs/mdocs/upov/en/two_46_prep/two_46_prep_exercise_2_explanation.pdf



Example 1: QL

Tomato mosaic virus / Tomato

1. Monogenic, dominant gene
2. Clear symptoms – no symptoms (sometimes hypersensitivity)

   Varieties are in general uniform, all plants in a tested variety show either clear or no symptoms. Light symptoms do not appear. Test result is very robust. 2 control varieties are enough, 1 susceptible, 1 resistant.
3. Clear mosaic symptoms: susceptible, note 1

   No symptoms or hypersensitivity: resistant, note 9
4. Conclusion: QL, **suitable for grouping**

Example 2: PQ

50. (*) (+)	VS/ VG	Resistance to *Bean common mosaic necrosis virus* (BCMNV)	Résistance au *Bean common mosaic necrosis virus* (BCMNV)	Resistenz gegen *Bean common mosaic necrosis virus* (BCMNV)	Resistencia al *Bean common mosaic necrosis virus* (BCMNV)		
PQ		absent	absente	fehlend	ausente	Dufrix, Flandria	1
		present with necrosis	présente avec nécroses	vorhanden mit Nekrose	presente con necrosis	Booster, Odessa	2
		present without symptoms	présente sans symptômes	vorhanden ohne Symptome	presente sin síntomas	Bizet	3

BCMNV / Bean

1. I-gene (dominant) and/or bc-genes (recessive)
2. Mosaic symptoms or necrosis or no symptoms.

   Varieties are in general uniform, all plants in a tested variety show either
   - mosaic symptoms (susceptible on both I-gene and bc-genes) or
   - no symptoms (resistance from bc-genes) or
   - they die because of necrosis (resistance from I-gene, expressed as hypersensitivity).

   Light symptoms do not appear. Test result is very robust. 3 control varieties are enough, 1 susceptible (mosaic), 1 resistant (no symptoms), 1 resistant (necrosis).
3. Conclusion: *PQ with states 1, 2, 3. Discontinuous states. Varies in more than one dimension.* ***Suitable for grouping (3 groups)***

Example 3: QN

45. (+)	Resistance to *Cucumber mosaic virus* (CMV)	Résistance au virus de la mosaïque du concombre (CMV)	Resistenz gegen Gurkenmosaikvirus (CMV)	Resistencia al virus del mosaico del pepino (CMV)		
QN	susceptible	sensible	anfällig	susceptible	Bosporus, Corona, Ventura	1
	moderately resistant	moyennement résistant	mäßig resistent	intermedia	Capra, Gardon, Verdon	2
	highly resistant	hautement résistant	hochresistent	alta	Naf, Picolino	3

***CMV* / Cucumber**

1. Polygenic
2. Observation scale is continuous, 5 levels

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

3. Conclusion: QN with 3 states, 1, 2 and 3. **And what about grouping? To be discussed**

To improve

Other resistance characteristics need discussions to improve:

- Indication QL, PQ, QN
- States
- Suitability for grouping
  - depending of the states of expression

Example 4: QL -> QN?

Phytophthora / Pepper

1. Polygenic
2. Continuous observation scale, development of symptoms in cm/week. Compare with standard varieties. All varieties with better resistance than moderately resistant control variety are resistant. Border is arbitrary, expected to be related to practice of growers.
3. At present QL with 2 states:
   1 (susceptible) and
   9 (resistant)
4. **To improve: QN**, states and controls at borders to be discussed

11.2	Observation scale	
	[1] absent	e.g. length increase > 0.8 cm/week
	[9] present (moderately resistant)	e.g. length increase ≥ 0.5 cm ≤ 0.8 cm/week
	[9] present (highly resistant)	e.g. length increase < 0.5 cm/week
11.3	Validation of test	on standards
11.4	Off-types	maximum 1 on 20 plants
12.	Interpretation of data in terms of UPOV characteristic states	QL Based on the stem necrosis increase compared to the standards. [1] susceptible: Jupiter, Yolo Wonder [9] moderately resistant: Favolor [9] resistant: Solario

Management of the states of QN characteristics

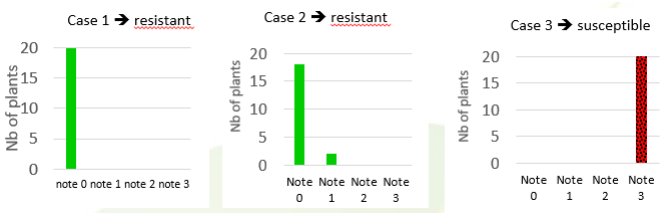
Can a QN characteristic be **suitable for grouping**? How to group with 3 groups?

Choice of states depends on the **combination of species / disease**

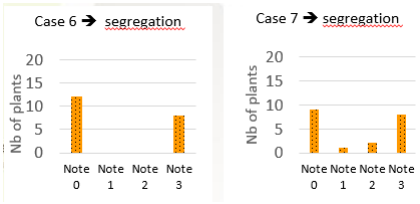
- 1-2-3: state 2 not distinct from 1 and 3 as such
- 1-2-5, 1-4-5: two states (1-2 or 4-5) are not distinct from the third one
- 1-3-9, 1-5-9, 1-7-9: all states are distinct
- 1-9 with a variety at the border deciding on a candidate scoring 1 or 9

To assess Uniformity for a QN characteristic (1)

- Easy to handle

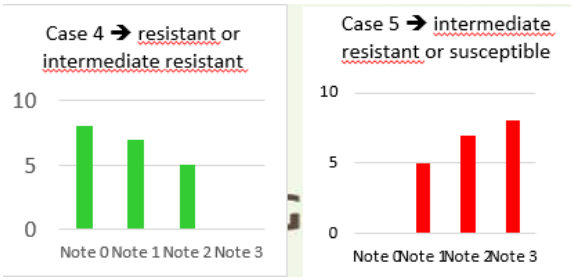


- Not uniform



To assess Uniformity for a QN characteristic (2)

Off-types? Relative uniformity?



Depends on the relation between the observation scale, the control varieties, and the characteristic states. To be discussed…

Summary

- Disease resistance in vegetables is important for breeders, growers, consumers.
- Resistance characteristics have an important value for
  - Grouping (management of the reference collection)
  - Description
  - Distinctness
- The characteristic needs
  - To be determined / updated based on plant/pathogen interactions, plant stage, races, isolates, calibrated regarding controls, knowledge on the different genetic backgrounds
  - Harmonized test method
- Technical Working Party has to determine whether a characteristic is fulfilling the UPOV criteria and which indication (QL, QN, PQ) and which states apply.
- Several test guidelines need an update.

[Annex IV follows]

ANNEX IV

CPVO · OCVV co funded project

Harmonization of resistance tests to diseases for DUS testing:

Harmores 3

UPOV meeting May 2019

crea Ctifl ESA Federal Plant Variety Office INIA nak tuinbouw nébih SASA UNIVERZITA PALACKÉHO V OLOMOUCI GEVES Expertise & Performance

Harmores 3 | Goal/deliverable | Results | Follow up

- **Three year project**: Part 1 June 2016 / June 2017 + Part 2 June 2017 /June 2019
- 7 **E0s** + 8 **ESA members** + 1 French **technical institute** involved
- Focus on intermediate resistances
  - More difficult than the previous projects
  - **Harmonized protocols** and **reproducible results** are of great concerns
    - Priorities in Tomato
      - *Meloidogyne incognita*
      - *Fusarium oxysporum* f. sp. *lycopersici* Race 0 (ex 1) and Race 1 (ex 2)
    - Priorities in Melon
      - *Fusarium oxysporum* f. sp. melonis race 1.2
      - *Fusarium oxysporum* f. sp. *melonis* race 2 (with also validation on races 0 and 1)
      - Powdery mildew (*Podosphaera xanthii*)
    - Priorities in Pea
      - *Erysiphe pisi*

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Harmores 3 | **Goal/deliverable** | Results | Follow up

Deliverables expected

- updated **bibliography** on host/pathogens chosen
- available **reference isolates** with **maintainers** laboratories
- available reference resistant, intermediate resistant and susceptible **controls**
- **culture conditions** defined for pathogens
- **test conditions** defined
- proposed **protocols** to update accordingly CPVO protocols

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Harmores 3 | **Goal/deliverable** | Results | Follow up

Phases of the project

Workshop and **Comparative tests**

scheduled on each pathogen

Task	Action	pathogen	Description	Year 1	Year 2	Year 3
1: Tomato	1.1	Fol:0; 1	Worshop notation scale	x		
	2.1	Mi	CT controls/panel	x		
	2.2	Mi	CT protocol comparison		x	
	2.2	Mi	Worshop notation scale		x	
	2.3	Mi	CT validation			x
2: Melon	1.1	Fom: 1.2	CT controls/panel	x		
	1.2	Fom: 1.2	CT protocol comparison		x	
	1.2	Fom: 1.2	Worshop notation scale		x	
	1.3	Fom: 1.2	CT validation			x
	2.1	Fom: 2	CT controls/panel	x		
	2.2	Fom: 2	CT protocol comparison		x	
	2.2	Fom: 2	Worshop notation scale		x	
	2.3	Fom: 2	CT validation			x
	3.1	Px	CT inoculation comparison	x		
	3.1	Px	Worshop notation scale	x		
	3.2	Px	CT protocol comparison		x	
	3.2	Px	Worshop notation scale		x	
	3.3	Px	CT validation			x
3: Pea	1.1	E spp	Collection of isolates	x		
	1.1	E spp	CT controls/panel	x		
	1.1	E spp	Common notation tests	x		
	1.2	E spp	CT controls/panel		x	
	1.3	E spp	CT protocol comparison			x

Harmores 3 | Goal/deliverable | **Results** | Follow up

Tomato/Mi

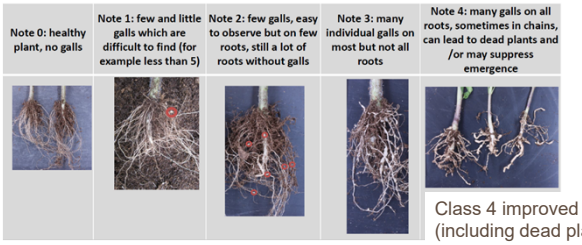
	Tested	Validated	Selected
Nb of controls	10	8	1S, 2 IR,1R
Nb of inoculation methods	5	2	1
Nb of climatic conditions	2	1	1
Notation scale	9	1	1

Inoculation method

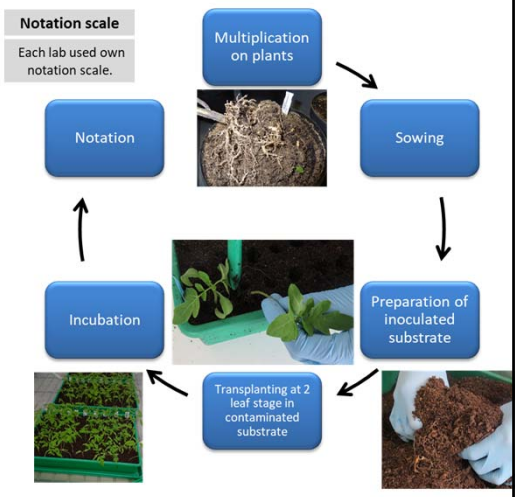
- **Validation** of a common method
  - Transplanting at 2 leaf stage in contaminated substrate
  - Addition of 10 non inoculated controls

Notation scale

- **Validation** of a common notation scale



Class 4 improved (including dead plants)

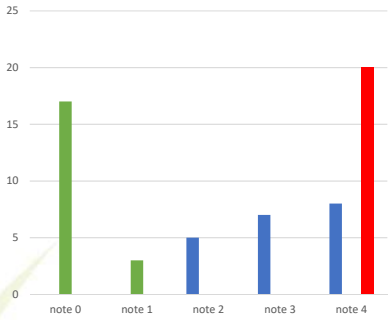


Harmores 3 | Goal/deliverable | **Results** | Follow up

Tomato/Mi

Decision rule

- To **validate controls**
- To validate varieties
- Written with examples to illustrate interpretations
  - and **including statistics** for unclear results



✓ Expected repartition of controls

- **Protocol validated** *with all aspects*, will be written for **October 2019**.

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Harmores 3 | Goal/deliverable | **Results** | Follow up

- **Tomato/Fol: 0 and 1**
  - **Controls**, **strains** and **inoculation method** harmonized (**Harmores 1**)
  - Some varieties with a certain level of intermediate resistance which is **not due to** a **genetic heterogeneity**.
    - ➢ *Not the same interpretation depending on labs*
  - **Comparative Tests of validation**
    - Controls (uncoded) and inoculation method from CPVO protocol
    - Isolate(s) : each lab chose the most robust under its own test conditions
    - Panel
  - **3 tests** on 20 plants:
    1. **Current CPVO protocol**
    2. Test on **big plants**, 3 labs, only on varieties on panel with **not clear cut compartment** and controls
    3. **Markers**, 3 labs
  - **Common notation** scale
  - **Common decision rule**

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Harmores 3 | Goal/deliverable | **Results** | Follow up

- **Tomato/Fol: 0** Comparative tests, standard test

Lab's interpretation

		Fol 0 - Standard test											
Variety	Expected	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 8	Lab 9	Lab 10	Lab 11	Lab 13	Lab 14	Lab 17
D	Not uniform	R	R	IR	HG	R	IR	R	R	HG	IR	R	S
E	Not uniform	R	R	HG	HG	IR	R	IR	HG	R	R	R	S
Marmande verte	S	S	S	S	S	S	S	S	S	S	S	S	S
Moneymaker	S	S	S	S	S	S	S	S	S	S	S	S	S
Cherry type control 1	IR	R	R	R	R	R	IR	R	R	R	R	R	R
Vispo	IR	R	R	IR	R	R	R	R	R	R	R	R	IR
A	IR	R	R	R	R	R	R	R	R	R	R	R	IR
B	IR	R	R	R	R	R	R	R	R	R	R	R	HG
C	IR	R	R	R	R	R	IR	R	R	R	R	R	R
G	IR	HG	R	R	HG	R?	IR	IR	S	HG	HG	HG	IR
Marporum x Marmande verte	R	R	R	R	HG	R	R	R	R	HG	R	R	R
Marporum	R	R	R	R	R	R	R	R	R	HG	R	R	R
Motelle	R	R	R	R	R	R	R	R	R	R	R	R	R

Pathostat interpretation

		Fol 0 - Standard test											
Variety	Expected	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 8	Lab 9	Lab 10	Lab 11	Lab 13	Lab 14	Lab 17
D	Not uniform	R	R	R	R	R	R	R	R	R	HG/S	R	HG/S
E	Not uniform	R	R	R	R	HG/S	R	R	HG/S	R	R	R	S
Marmande verte	S	S	S	S	S	S	S	S	S	S	S	S	S
Moneymaker	S	S	S	S	S	S	S	S	S	S	S	S	S
Cherry type control 1 = G	IR	R	R	R	R	R	R	R	R	R	R	R	R
Vispo	IR	R	R	R	R	R	R	R	R	R	R	R	R
A	IR	R	R	R	R	R	R	R	R	R	R	R	HG/S
B	IR	R	R	R	R	R	R	R	R	R	R	R	HG/S
C	IR	R	R	R	R	R	R	R	R	R	R	R	R
G	IR	R	R	R	R	R	R	R	S	R	HG/S	HG	HG/S
Marporum x Marmande verte	R	R	R	R	R	R	R	R	R	R	R	R	R
Marporum	R	R	R	R	R	R	R	R	R	R	R	R	R
Motelle	R	R	R	R	R	R	R	R	R	R	R	R	R

- ➢ More varieties **statistically judged not different** than **controls**
- ➢ E and G also tested **on big plants**
- ➢ Discussion on **Uniformity** (HG) and in many cases **not validation of the test on controls**

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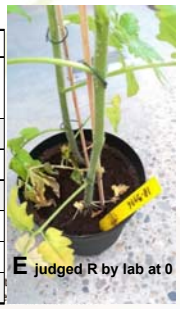
Harmores 3 | Goal/deliverable | **Results** | Follow up

- **Tomato/Fol: 0** Comparative Test, big plants

		Fol 0 - Test on big plants		
Variety	Expected	Lab 3	Lab 5	Lab 6
E	Not uniform	IR	R	R
Marmande verte	S	S	S	S
G	IR	IR	R	R
Marporum x Marmande verte	R	R	R	R
Marporum	R	R	R	R
Motelle	R	R	R	R

✓ Good correlation between labs
✓ All varieties judged as IR or R compared to controls

Symptom on vessels						
Seed sample code	Repetition	Precise number of plants in each category/notation scale: 0 : no symptoms	1: brown vessel above the cotyledon	2: yellowing and/or wilting on leaves.	Total plants tested	Interpretation
Marmande verte	/1	0	1	9	10	S
	/2	1	2	9	12	
Marporum x Marmande verte	/1	9	0	1	10	R
	/2	11	1	0	12	
Marporum	/1	9	0	1	10	R
	/2	10	1	0	11	
Motelle	/1	10	0	0	10	R
	/2	12	0	0	12	
301	/1	4	0	5	9	IR
= E	/2	7	1	4	12	
313	/1	3	0	7	10	IR
= G	/2	7	0	5	12	



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Harmores 3 | Goal/deliverable | **Results** | Follow up

- **Tomato/Fol: 0** Comparative Test, marker tests for ***I2*** gene (confers resistance to Fol: 0 and 1)

	Lab 3					Lab 5					Lab 6				
Variety	Biotest		Homo I2	Hete I2	Homo S	Biotest		Homo I2	Hete I2	Homo S	Biotest		Homo I2	Hete I2	Homo S
D	27-1-2-0	R			20										
E	25-4-1-0	R		1	20						15-0-5-0	IR			4
Marmande verte	1-0-14-15	S			28	1-0-6-13	S			7	0-0-20-0	S			4
Moneymaker	2-0-4-16	S			13										
Cherry type control 1 = G	27-1-2-0	R			18	18-0-2-0	R								
Vispo	24-0-0-0	R		18	1										
A	30-0-0-0	R	2	18											
B	29-1-0-0	R		20											
C	30-0-0-0	R	9	11											
G	25-4-1-0	R			18					5					
Marporum x Marmande verte	28-1-1-0	R			30	18-0-2-0	HG			5					
Marporum	28-0-1-0	R			30	20-0-0-0	R			10	16-3-3-0	R			4
Motelle	30-0-0-0	R	17	1		20-0-0-0	R								

➢ **2 varieties R** in phenotype but S markers: *certainly not I2 gene involved*
➢ 2 varieties tested **genotypically heterogeneous** (homozygous and heterozygous *I2*) but with a **homogeneous phenotype** predicted (resistant), confirmed by biotest.

10

Harmores 3 | Goal/deliverable | **Results** | Follow up

Tomato/Fol: 0 Comparative Test, marker tests for ***I2*** gene (confers resistance to Fol: 0 and 1)

2019 LIMITS:
- Only 3 labs involved,
- with sometimes a very reduced number of plants

Conclusion: not enough data and inconsistent results between labs to validate the markers ***at this stage***

Additional tests (fall 2019) are planed:
- including 20 plants/lab
- to allow *if possible* some validations

Variety	Lab 3 Biotest		Lab 3 Homo I2	Lab 3 Hete I2	Lab 3 Homo S	Lab 5 Biotest		Lab 5 Homo I2	Lab 5 Hete I2	Lab 5 Homo S	Lab 6 Biotest		Lab 6 Homo I2	Lab 6 Hete I2	Lab 6 Homo S
D	27-1-2-0	R			20										
E	25-4-1-0	R		1	20						15-0-5-0	IR			4
Marmande verte	1-0-14-15	S			28	1-0-6-13	S			7	0-0-20-0	S			4
Moneymaker	2-0-4-16	S			13										
Cherry type control 1 = G	27-1-2-0	R			18	18-0-2-0	R								
Vispo	24-0-0-0	R		18	1										
A	30-0-0-0	R	2	18											
B	29-1-0-0	R		20											
C	30-0-0-0	R	9	11											
G	25-4-1-0	R			18					5					
Marporum x Marmande verte	28-1-1-0	R			30	18-0-2-0	HG			5					
Marporum	28-0-1-0	R			30	20-0-0-0	R			10	16-3-3-0	R			4
Motelle	30-0-0-0	R	17	1		20-0-0-0	R								

- **2 varieties R** in phenotype but S markers: *certainly not I2*
- 2 varieties tested **genotypically heterogeneous** (homozygous and heterozygous *I2*) but with a **homogeneous phenotype** predicted (resistant), confirmed by biotest.

11

Harmores 3 | Goal/deliverable | **Results** | Follow up

Tomato/Fol: 1 Comparative Test on marker tests for ***I2*** gene (confers resistance to Fol: 0, 1)

Variety	Lab 3 Biotest		Lab 3 Homo I2	Lab 3 Hete I2	Lab 3 Homo S	Lab 5 Biotest		Lab 5 Homo I2	Lab 5 Hete I2	Lab 5 Homo S	Lab 6 Biotest		Lab 6 Homo I2	Lab 6 Hete I2	Lab 6 Homo S
Marmande verte	0-0-0-30	S			30						0-0-30-0	S			4
Marporum	0-0-1-29	S			30						0-0-16-14	S			4
Moneymaker	0-0-0-30	S			15						0-0-26-2	S			4
Galaxy	11-0-0-0	R		9	1	17-0-3-0	HG		5	1	19-7-4-0	IR		4	
Fol Harmo	24-1-4-0	HG		19							11-8-11-0	IR?		4	
K	2-0-0-12	HG	1		5						0-3-21-6	S			4
L	18-0-3-3	HG		12	8						30-0-0-0	R		4	
Motelle x Marmande verte	26-1-2-0	R		28		18-0-2-0	HG		9		14-15-1-0	IR		4	
Cherry type control 2 = H	26-0-2-0	R	20			15-1-4-0	HG	1	8	1	21-6-2-0	R	4		
H	24-2-4-0	IR	13	7							13-11-6-0	IR	4		
I	19-5-5-1	IR		20		17-2-1-0	HG			4	22-7-1-0	R		4	
J	9-2-10-0	HG	12	6		4-0-8-8	S			10	12-6-7-0	IR	4		

- varieties tested **genotypically heterogeneous** (homozygous and heterozygous *I2 or homozygous S and heterozygous I2*)
- Differences between labs (but sometimes very reduce number of tested plants)
- S plants not detected by some lab.

Conclusion: not enough data and inconsistent results between labs to validate the markers ***at this stage***

Additional tests (fall 2019) are planed:
- including 20 plants per lab
- to allow if possible some validations

Harmores 3 | Goal/deliverable | **Results** | Follow up

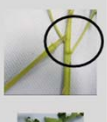
Tomato/Fol: 0 and 1

Notation scale

- Validation of a **common notation scale**

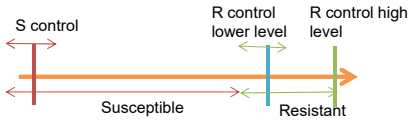
Decision rule

- To validate **controls**
- To validate varieties
- Written with **examples to illustrate, interpretation** and with **statistics** in case of unclear results

Non-inoculated plants	Class 0	Class 1	Class 2	Class 3
Varieties must be compared to the non-inoculated plants especially for the growth reduction criteria.	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 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.				

Controls

- **Lower level of R** not distinct enough in all tests to define a new level
- Decision to add a **control** used as threshold for R, so 2 R levels for R controls



- Protocol validated with all aspects **except markers**, will be written for October 2019
- **Web meeting** in 2019 to set up a test plan to validate markers

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Harmores 3 | Goal/deliverable | **Results** | Follow up

Melon/ *Podosphaera xanthii*

- 1 **inoculation method** validated: on whole plant
- **Stage** and **number of plant** validated
- **S and R controls** validated
- **Common decision rule** to validate controls and interpret varieties defined (quantitative analysis with Disease Index)
- **Notation scale** to be extended with an **additional note** (to validate during ISF ring test under preparation)
- Skype meeting to work on **interpretation based on repartition of notes**

- Define then if protocol ready to be written

Melon/*Fusarium oxysporum* f. sp. melonis race 1.2

- 2 **inoculation** methods
- 2 states S and IR. IR with 3 **controls**
- **Statistics**

- **Protocol validated** with all aspects, will be written for October 2019

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Harmores 3 | Goal/deliverable | **Results** | Follow up

- **Melon/ *Fusarium oxysporum* f. sp. *melonis* race 2 (with also validation on races 0 and 1)**
  - 1 **inoculation method** validated: soaking and transplanting
  - 2 states S and R. 2 **controls**

- **Protocol validated** with all aspects, will be written for October 2019

- **Protocol validated** with all aspects, will be written for October 2019

- **Pea/ *Erysiphe pisi***
  - 2 **inoculation methods** validated: spraying and sprinkling
  - 2 states S and R. **2 controls** for **vegetable peas**, **2** for **agricultural peas**
  - 1 **isolate** chosen

		Controlled conditions			
Variety	**Expected comportment**	**Lab 2**	**Lab 3**	**Lab 6**	**Lab 7**
Cabree	Susceptible	S	S	S	S
Ottoman	Susceptible	S	S	S	S
Aladin	Susceptible	S	S	S	S
JL2302	Resistant	R	R	R	R
Ema	Resistant	R	R	R	R
Vivaldi	Resistant	R	R	R	R
Alezan	Resistant	R	R	R	R
Sugar Bon	Resistant	R	R	R	R

Harmores 3 | Goal/deliverable | Results | **FOLLOW UP**

- Report for CPVO postponed to **15/09/2019**
- **Follow up actions**
  - Melon / Powdery mildew (*Podosphaera xanthii*): ISF **ring test** for notation scale
  - **Test plan for validation of markers** for Tomata / *Fusarium oxysporum* f. sp. *lycopersici* Race 0 (ex 1) and Race 1 (ex 2)
- **Protocols**
  - **To be defined** after web meeting (fall 2019)
    - Melon / Powdery mildew (*Podosphaera xanthii*)
  - **To be written** and sent to CPVO for October:
    - Tomato / *Meloidogyne incognita*
    - Tomato / *Fusarium oxysporum* f. sp. *lycopersici* Race 0 (ex 1) and Race 1 (ex 2)
    - Melon / *Fusarium oxysporum* f. sp. melonis race 1.2
    - Melon / *Fusarium oxysporum* f. sp. *melonis* race 2 (with also validation on races 0 and 1)
    - Pea / *Erysiphe pisi*

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

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[Annex V follows]

Disease resistance in Vegetables:

What does the European industry do in terms of claims?

UPOV TWV 53, May 2019, South Korea

Christophe ROUILLARD, European Seed Association Technical Manager

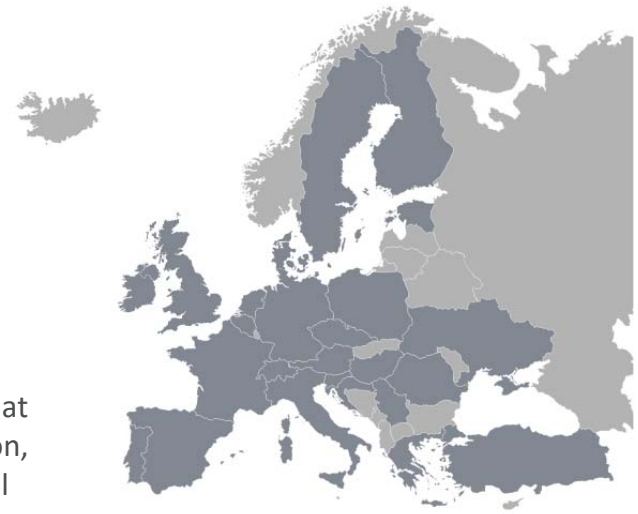
Agenda

- **European Seed Association**
  - ✓ A brief introduction
- **Harmonisation in Resistance Terminology in the Vegetables sector**
  - ✓ What does the industry do in Europe and for which purpose?

European Seed Association: a brief introduction

- **Single** voice of the European seed sector: 38 national seed associations, 40 direct companies, 29 associates
- The role is to:
  - ✓ **Inform**
  - ✓ **Represent**
  - ✓ **Lobby**

  on **all** seed related issues
- Represents the European seed sector at the **European institutions**: Commission, Parliament, Council.. and International organisations



Priority issues for the European seed sector

Plant Breeding Innovation

Intellectual Property

Research & Development (Funding)

Trade & Plant Health

Biodiversity, Access Benefit Sharing (ABS)

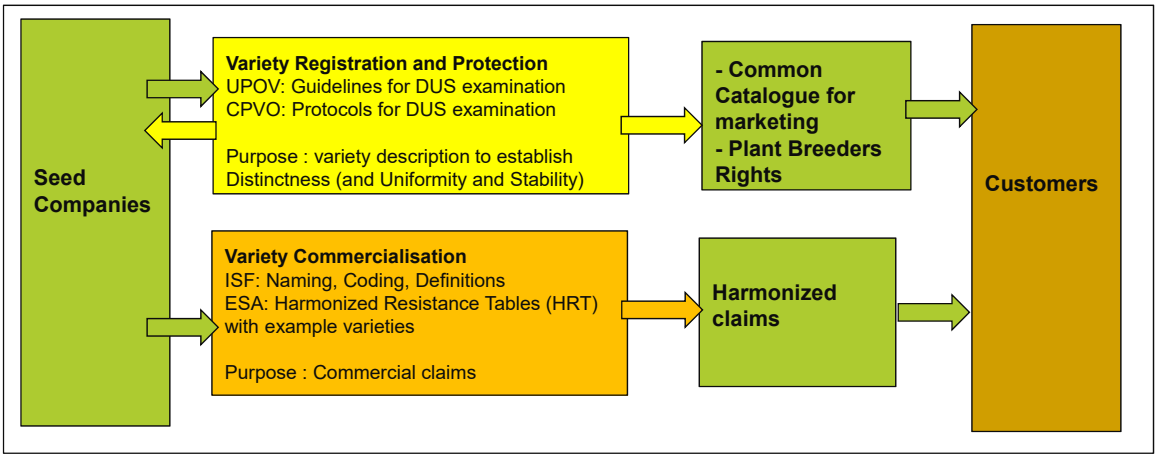
Seed Treatment

Harmonisation in Resistance Terminology in the Vegetables sector

What does the industry do in Europe and for which purpose?

ESA

Disease resistance testing : an overview

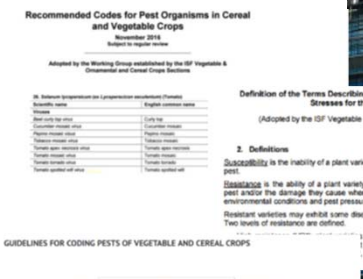


Varieties compliant to multiple standards before marketing:

- mandatory (DUS) or Commercial claims

Harmonisation in Resistance Terminology in the Vegetables sector– development at EU level

- European breeders rely on activities conducted within ISF to have :
- **Industry alignement on the use of consistent terminology in the communication to customers:**
  - *Pathogen naming*
  - *Pathogen coding*
  - *Definitions*
  - *Guidelines for coding*
  - *Characterized races*


Recommended Codes for Pest Organisms in Cereal and Vegetable Crops
November 2016
Subject to regular review

Definition of the Terms Describing the Reaction of Plants to Pests' and Abiotic Stresses for the Vegetable Seed Industry
(Adopted by the ISF Vegetable and Ornamental Crops Section in June 2012)

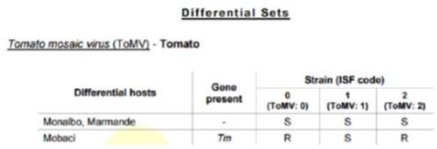
2. Definitions

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

Resistance is the ability of a plant variety to restrict the growth and development of a specified pest and/or the damage they cause when compared to susceptible plant varieties under similar environmental conditions and pest pressure.

Resistant varieties may exhibit some disease symptoms or damage under heavy pest pressure. Two levels of resistance are defined.

GUIDELINES FOR CODING PESTS OF VEGETABLE AND CEREAL CROPS


Differential Sets

Tomato mosaic virus (ToMV) - Tomato

Differential hosts	Gene present	Strain (ISF code) 0 (ToMV: 0)	1 (ToMV: 1)	2 (ToMV: 2)
Monalbo, Marmande	-	S	S	S
Mobaci	*Tm*	R	S	R

Harmonisation in Resistance Terminology in the Vegetables sector– development at EU level

- ***Company claims*** *on the level of resistance in a variety to a pathogen are based on tests carried out with well-characterized isolates of the pathogen in controlled environmental conditions.*
- *This resistance may be effective against all or some biotypes, pathotypes, races or strains of the pathogen. However, pathogens are known to develop and form new biotypes, pathotypes, races or strains that can cause damage to plants that remain unaffected by the original form of the pathogen.*

- Need for the seed industry **to communicate to the vegetable value chain in a coordinated manner** :
- Creation in 2008 of a Working Group within ESA (Section for Vegetables) on „**Harmonisation in Resistance Terminology**“

Harmonisation in Resistance Terminology in the Vegetables sector– development at EU level

- Current ESA Working Group composed of experts in phytopathology from vegetables seed companies representing expertise on all crops

- Objective of the WG

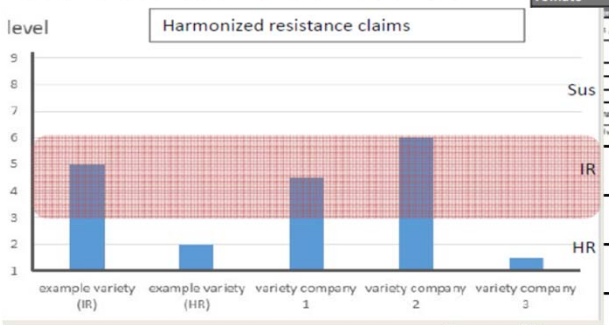
  To provide the vegetable industry ***with clear and consistent communication*** *on disease resistance in vegetable crop varieties*

  - Aim of this Group is not to discuss disease resistance used as characteristic to differentiate varieties

ESA messaging and advocacy

- Activites conducted by the ESA Working Group

  → put together information on the strain/race of a pathogen to which commercial or other varieties are resistant and the level of resistance **associated with specific example varieties**



- Resistance Harmonization Tables published on the ESA website

CODE FOR RESISTANCE HARMONIZATION

ESA_16.0794
October 2016

The ESA Code for resistance harmonisation has been designed by ESA to improve transparency to vegetable growers.

Vegetable seed companies are recommended to make use of the ESA Code in their catalogues, on their websites and in all other forms of product information

Tomato

	Ua	44+49+54+73 +108	HR	Early Gallatin
	Ua	41+44+47+53 +54	HR	Mex 309
	Ua	38+49+53+67 +108	HR	Mex 235
Uromyces appendiculatus	Ua	41+44+47+49 +53+54+67+7 3	HR	BelMiDak-RR-5
Uromyces appendiculatus	Ua	90	IR	Teresa

Take home messages

ESA WG Harmonized Resistance Terminology

- Industry alignment on harmonized resistance claims in the communication to customers

Disease resistance in vegetables:

- Important goal for breeders
- Use of agreed terminology is crucial not only in DUS tests (aimed at differentiate varieties) but also in commercial claims

Thanks!

CONTACT US

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B 1000 Brussels

T. +32 (0)2 743 28 60

secretariat@euroseeds.eu

[Annex VI follows]

International Seed Federation
Seed is Life

ISF Working Group Disease Resistance Terminology

UPDATES

UPOV TWV Seoul, 20-24 May 2019

Content

- ISF in General
- Working Group Disease Resistance Terminology
- Guideline for nomination novel races and strains
- Full revision of Differential Tables
- New Projects

ISF's roots

What is ISF?

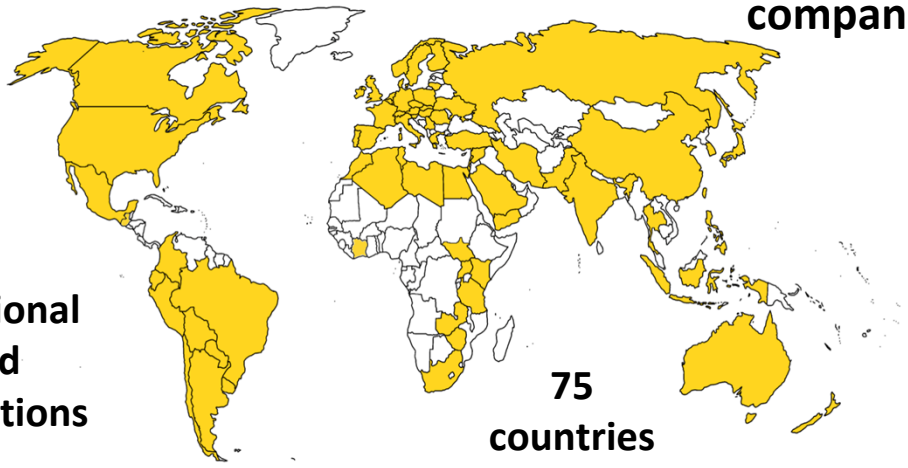
- "Voice of the global seed industry"
- Non-governmental, non-profit making organization
- Recognizes its members' contributions to food security and sustainable agriculture.

Role of ISF

- **Represents interests** of the seed industry at a global level
- **Facilitates free movement** of seed within a fair framework
- **Promotes IP rights** for seeds, plant varieties and technologies
- **Informs** members of developments in industry, and in the international regulatory environment

ISF in figures

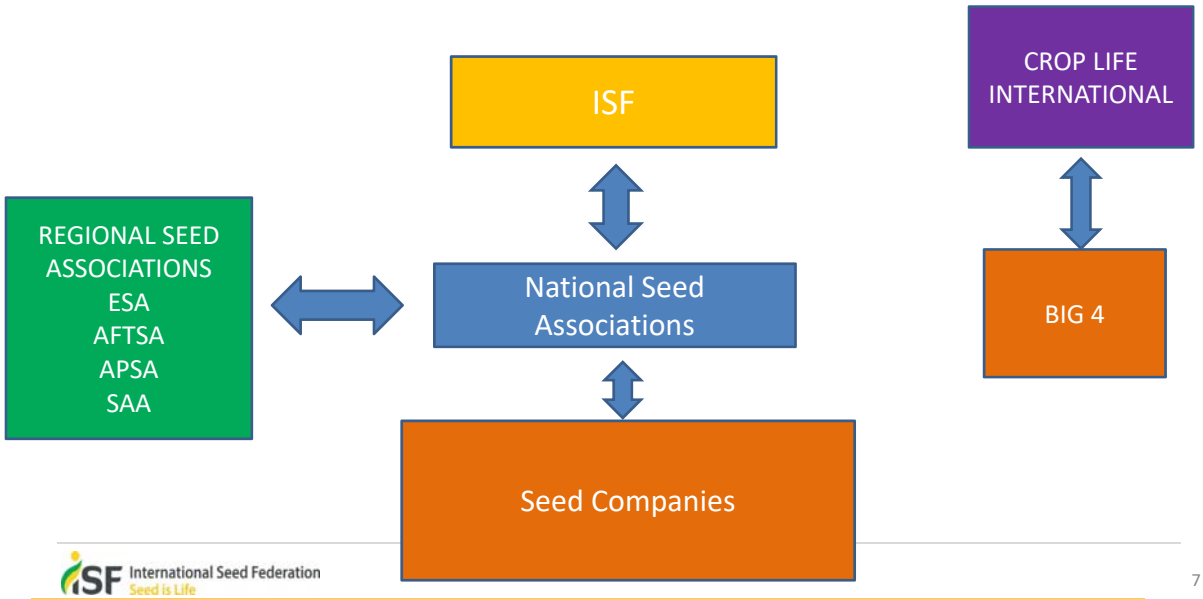
7500+ companies



58 National Seed Assoications

75 countries

Structure of representation of the seed industry



ISF Vision and Mission

ISF Vision

"A world where the best quality seed is accessible to all, supporting sustainable agriculture and food security."

ISF Mission

"To create the best environment for the global movement of seed, and promote plant breeding and innovation in seed"

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Strategic Objectives

ISF Strategic Objectives 2016-2020

- **Innovation**
  - Consistent policies for products of plants developed through latest plant breeding methods
- **Movement of seed**
  - Harmonization of frameworks for phytosanitary measures
  - Harmonization of regulations for seed applied technologies
  - Seed certification schemes and seed quality assurance systems
- **Intellectual Property Rights**
  - Simplification of procedures and cooperation between countries for PVP
  - Support members with implementing IP rights in their countries

ISF Strategic Objectives 2016-2020

- **Biodiversity**
  - To promote the International Treaty as the preferred tool to administer Plant Genetic Resources for Food and Agriculture (PGRFA)
- **Engagement**
  - To engage with our members to strengthen cooperation
  - To engage with all stakeholders in the value chain to foster cooperation
  - To raise awareness and reputation of the seed industry and the benefits it brings to a global society.

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Working Group Disease Resistance Terminology

Background and scope

Disease resistance

- A major objective when breeding for new vegetable varieties.
- Disease resistance needs to be carefully described.
- One of the means of differentiating new varieties from those already on the market.

Terminology with respect to disease resistance

- Important to promote the international use of consistent terminology.
- Provide a uniform message to the market to avoid customer confusion.
- Avoidance of product liability and eventual claims.

Tasks of the Working Group

- Codes pathogens for which companies claim disease resistance for their varieties
- Promotes harmonized terminology across the industry to avoid any liability due to miscommunication
- Develops host differentials
- Establishes validated procedures based on peer-reviewed scientific publications and industry practices to identify pathogen races/strains

Composition of the WG

Name (company)	Representing
Valerie Grimault (GEVES)	**Chair** MATREF and GEVES (FR)
Wim Sangster(Naktuinbouw)	Naktuinbow (NL), IBEB (Europe/US)
Eelco Gilijamse (Rijk Zwaan)	Plantum (NL) Isolates GroupPlantum (NL) WG Disease Resistance
Ton Allersma (Bayer)	ESA WG HRT (Europe)
Vacancy	UFS WG DRT (FR)
Phyllis Himmel (CPPSI) Philip Brown (Sakata)	CPPSI (US)
Pieter van Poppel (BASF)	IWGP/IBEB
Cristina Moyano (INIA)	INIA (ES)

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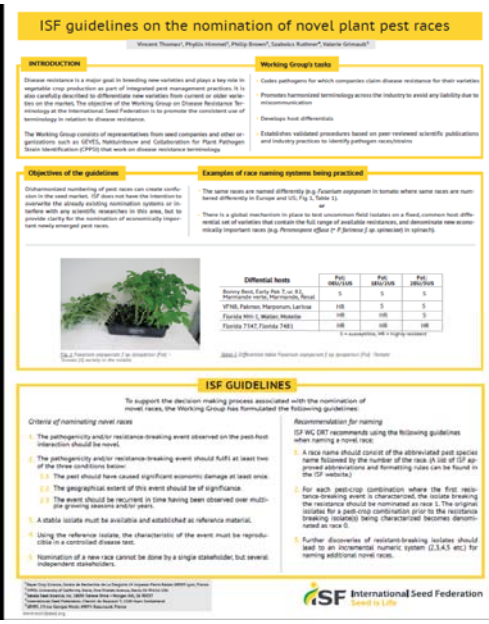
ISF guidelines on the nomination of novel plant pest races

Implementation of the Guideline


ISF guidelines on the nomination of novel plant pest races

INTRODUCTION

Working Group's tasks

Objectives of the guidelines

Examples of race naming systems being practiced

Differential hosts

ISF GUIDELINES

Criteria of nominating novel races

Recommendation for naming

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Full revision of the Differential tables

- Short introduction if needed and more information in the footnote
- Always put ISF code, remove pathotype
- Use the term differential host and not variety consequently
- Use HR= highly resistant and not R
- *: not yet tested/under review by the members of ISF DRT group,
- Include the gene when it is informative,
- Use references that are just enough to support table, literature in broad sense, can be CPVO reference

Update on differential tables revisions

Reviewed

- ✓ Bean Halo Blight (Psp)
- ✓ Bean-Anthracnose (Cl)
- ✓ Bean-BCMV BCMNV
- ✓ Bean-Fusarium Wilt (Fop)
- ✓ Brassica oleracea-clubroot (Pb)
- ✓ Cabbage Fusarium yellows (Foc)
- ✓ Cucumber Fusarium Wilt (Foc)
- ✓ Lettuce Lettuce mosaic virus (LMV)
- ✓ Pea Ascochyta pisi (Aps)
- ✓ Pea Near Wilt (Fop)
- ✓ Pepper Potyviruses
- ✓ Pepper Tobamoviruses
- ✓ Pepper Bacterial leaf spot (Xcv)
- ✓ Spinach Downy mildew (Pfs)
- ✓ Tomato - Tomato Mosaic Virus
- ✓ Tomato Fusarium Wilt (fol)
- ✓ Tomato Tobamoviruses

Work in progress

- Lettuce Fusarium wilt (Fol)
- Tomato Leaf mold (Ff)
- Watermelon Fusarium wilt
- Melon Fusarium wilt (Fom)

New

- Melon – Melon Necrotic Spot Virus (MNSV)
- Celery - Fusarium yellows and wilt (Foa)
- Lettuce- Lettuce leaf aphid (Nr)
- Tomato/Pepper – Tomato spotted wilt virus (TSWV)

To be deleted

- Bean Rust (Ua)
- Root knot nematodes

Differential table (New Feature)

Differential hosts	Fol: 0EU/1US *	Fol : 1EU/2US*	Fol : 2EU/3US*
Bonny Best, Early Pak 7*, uc 82, Marmande verte*, Marmande*, Resal	S	S	S
VFN8*, Pakmor*, Marporum*, Larissa	HR	S	S
Florida MH-1*, Walter*, Motelle*	HR	HR	S
Florida 7547*, Florida 7481*	HR	HR	HR

S = susceptible; HR = highly resistant
*differential hosts and isolates that are used by the seed sector

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New Project Example

Powdery Mildew on Melon

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The ISF DRT WG Melon Px Initiative

- The aim is to develop a manageable differentiating hosts, assemble relevant Px races and protocols that would lend themselves to routine disease resistance testing that supports claims of resistance
  - International in scope
  - Based on the major melon Px resistance genes
  - Candidate differentials and melon Px races were proposed based on presented data
  - Incorporate the septet coding system described by Lebeda and Jaunet
- Our focus is on the economically impactful races of melon Px against which claims of disease resistance are made: 1, 2, 3.5 and 5

Criteria for Melon Px Differential Hosts

- Major resistance genes
- Not redundant with other differentials in response to Px
- Capacity to differentiate
- Available
- Easy to increase
- No necrotic reaction
- Good correlation between leaf disc and seedling test
- Consistent results between labs
- *Cucumis melo*

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Candidate Melon Px Differential Hosts

Vedrantais
PMR45
PMR5
Edisto47
RIL 1 (PI414723)
PI482420
RIL 4 (PI414723
SVI105
WMR29
PI124112
PI313970
Ames 31282

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Next Steps:

Seed increases almost completed

Validation test plan was confirmed

First Round of Validation testing begins in 2019

- Candidate differentials
- Candidate reference strains
- 12 US and EU labs
- Evaluate protocols

Meet to discuss results and rating interpretations

Decide when to run second round of testing to confirm results

Seed is Life

[End of Annex VI and of document]