UPOV

International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables

TWV/54/6 Rev.

Fifty-Fourth Session
Brasilia, Brazil, May 11 to 15, 2020

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USE OF DISEASE RESISTANCE CHARACTERISTICS

Document prepared by the Office of the Union

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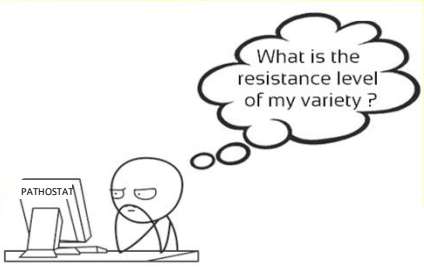
This document contains presentations to be made at the fifty-fourth session of the Technical Working Party for Vegetables (TWV):

Annex I "Data processing for disease resistance characteristics: the Pathostat application", prepared by an expert from France;

Annex II "Disease resistance tests on *Solanum sisymbrifolium*, *S. torvum* and *S aethiopicum*: tomato and eggplant rootstoks - Italian laboratory experience", preapred by an expert from Italy.

[Annexes follow]

Data processing for disease resistance characteristics: the Pathostat application



FR Presentation at the **UPOV - TWV 54**

Web-meeting from
2020-05-11 to 2020-05-15

GEVES
Expertise & Performance

Pathostat – Veg Project (2016 – 2019)

- A French Ministry of Agriculture project (2016-2019)

Liberté • Égalité • Fraternité
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GEVES Expertise & Performance
Groupe d'Étude et de contrôle des Variétés Et des Semences

INRAE

Ctifl

ufs UNION FRANÇAISE DES SEMENCIERS

GAUTIER semences

Vilmorin

MIKADO

HM•CLAUSE

SAKATA

PASSION in Seed

- Context : - For a better management of the **sustainability of genetic resistances** use of **polygenic or intermediate forms of resistance**

- DUS assessment of varieties *more complicated.*

Variety R Variety IR Variety S
Lettuce / *Fusarium* oxysporum f. sp. *lactucae*

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Pathostat – Veg Project (2016 – 2019)

- Thanks to this project:
  - Select **statistical tests** applicable to **quantitative results of bio-tests**
  - Develop a **simple and robust application** to support **expert analysis**
  - For DUS:
    - To assess the uniformity of the characteristic
    - Identify the distinction (or not) of an application regarding the behavior of official reference varieties, based on non qualitative data, to allow the description of the application.

- Purposes :
  - **Improvement and harmonization** of decision rules on the **interpretation** of biotest results
  - Improvement of **variety assessment**
    - Better correlation between applicants' claims and official tests

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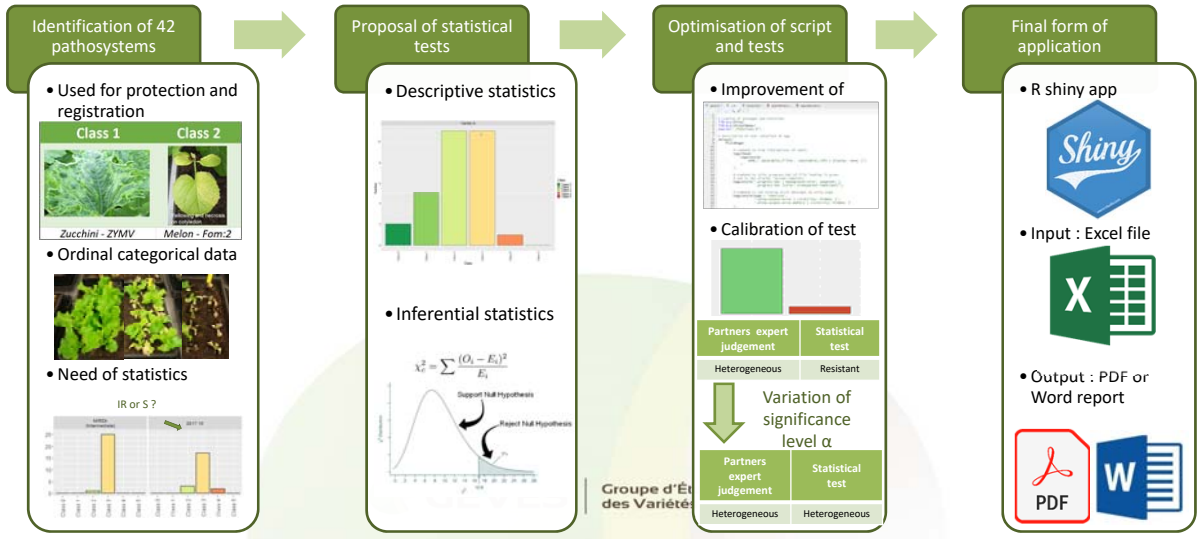
Pathostat – Veg Project (2016 – 2019)

- 3 years French Ministry project on vegetables
- Objective : creation of a statistic tool, a simple and robust application to support expert analysis



PATHOSTAT application

- **Free hosting and open access on GEVES website**
  - ⇨ https://pathostat.geves.fr
- **Application is secure**
  - ⇨ Data are not saved within application
- **User manual**
  - ⇨ Exists in English and French
- Application has **2 functions** :
  - ⇨ The respect of the regulatory framework and official protocol
  - ⇨ Adaptability for methodology or research

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Selection and parametrization of the biotest → Comparison of distribution between repetitions → Study of the S/R of the varieties

Bean - *Colletotrichum lindemuthianum* race 6	**Lettuce** - *Bremia lactucae*	35 couples available	**Tomato** - *Fusarium oxysporum* f.sp. *lycopersici* race 0
Bean - *Colletotrichum lindemuthianum* race Kappa	**Lettuce** - *Fusarium oxysporum* f.sp. *lactucae*	**Pea** - *Ascochyta pisi*	**Tomato** - *Fusarium oxysporum* f.sp. *lycopersici* race 1
Bean - *Pseudomonas savastanoi* pv *phaseolicola* race 6	**Melon** - *Fusarium oxysporum* f. sp. *melonis* race 0	**Pea** – BYMV	**Tomato** - *Fusarium oxysporum* f.sp. *lycopersici* race 2
Cabbage - *Fusarium oxysporum* f. sp. *conglutinans*	**Melon** - *Fusarium oxysporum* f. sp. *melonis* race 1	**Pea** – *Erysiphe pisi*	**Tomato** - *Fusarium oxysporum* f.sp. *radicis lycopersici*
Cucumber - *Cladosporium cucumerinum*	**Melon** - *Fusarium oxysporum* f. sp. melonis race 1.2	**Pea** - *Fusarium oxysporum* f.sp *pisi* race 1	**Tomato** - *Meloidogyne incognita*
Cucumber – CMV	**Melon** - *Fusarium oxysporum* f. sp. *melonis* race 2	**Pepper** - *Meloidogyne incognita*	**Tomato** - *Pseudomonas syringae* pv. *tomato*
Cucumber - *Corynespora cassiicola*	**Melon** – MWMV	**Zucchini** – CMV	**Tomato** - *Pyrenochaeta lycopersici*
Cucumber – CVYV	**Melon** - *Podosphaera xanthii* race 1, 2, 3, 5, 3-5	**Zucchini** – WMV	**Tomato** - *Stemphylium* spp.
Cucumber - *Podosphaera xanthii*	**Melon** – ZYMV	**Zucchini** – ZYMV	**Tomato** - *Verticillium dahlia*

1- Selection of the pathosystem
- with repetitions?
- download of the spreadsheet

2- Loading of resistance test raw data

3- Minimum threshold number
- It depends on pathosystem
- Reference threshold number is automatically selected
- Option to change the value of the minimum threshold

Test of the minimum number

4- Selection of controls

Selection and parametrization of the biotest | **Comparison of distribution between repetitions** | Study of the S / R of the varieties

- **Stacked histogram**
  - To compare graphically **distribution of symptoms** between repetitions for each variety
  - **Highlights differences** between repetitions

- **Homogeneity test of replications**

- To study the distribution of symptoms **between repetitions**
  - **All varieties are pooled together**
  - Test is performed **whatever the number of classes and repetitions**
  - **Highlights any problems in experimental protocol**
  - Option to merge classes

Chi-squared test (χ^2 test)

- **Homogeneity test of replication per variety**

- To compare distribution **between repetitions** of **each variety**
  - Preferred to χ^2 test because: *small number* of each repetition and *predictable imbalance* of the expected value

Fisher's exact test

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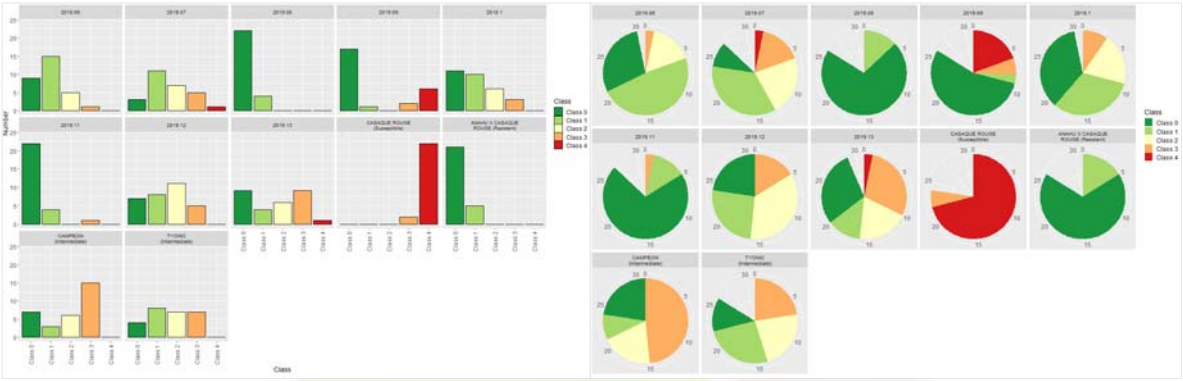
Selection and parametrization of the biotest | **Comparison of distribution between repetitions** | **Study of the S/R of varieties**

- **Histogram / Pie chart:**

which allow to view the distribution of symptoms for each variety



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Selection and parametrization of the biotest | Comparison of distribution between repetitions | Study of the S/R of varieties

Hierarchical Cluster Analysis:

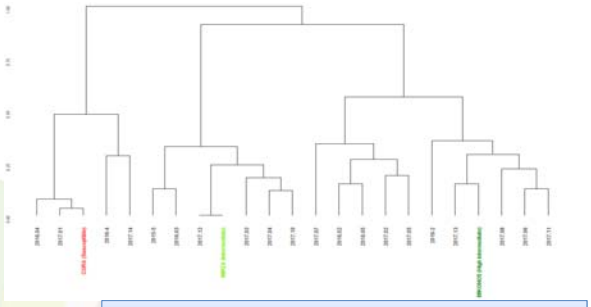
- *Principle of the method*: increase **intraclass homogeneity** and increase **interclass heterogeneity**

- with a **Criterion of similarity** : relative frequency of affiliation to each class of symptom
- based on **Euclidean distance**
  - ➢ Production of a Dendrogram (classification tree)

Using 5 agglomerative methods :

- Single-linkage
- Complete-linkage
- Ward.D2
- UPGMA
- UPGMC

To produce a **Cophenetic correlation coefficient**



⚠ Does not take into consideration the position of symptom classes

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Selection and parametrization of the biotest | Comparison of distribution between repetitions | Study of the S/R of varieties

Decisions

– **Heterogeneity rules** (view of the distribution of symptoms / each var.)

A variety is proposed HETEROGENEOUS if there are:

Plants in both extreme classes within the limit of the **tolerated number of off-types**

No plant in at least one of the intermediate classes

→

The tolerated number of off-types of extreme classes depends on:

Species

Number of plant in extreme classes of control

– **Susceptibility / resistance level of varieties** decided **on comparison with controls**

Model which **compare** each variety with **each control**: Cochran- Armitage test

It is considered as an enhancement of the χ^2 test, because it allow considering the **general tendancy.**

Hypothesis of the Cochran-Armitage test for trend according to controls

- H_0: susceptibility level of variety is higher or equal to control - H_1: susceptibility level of variety is higher or equal to control	- H_0: resistance level of variety is higher or equal to control - H_1: resistance level of variety is higher or equal to control

Susceptible control

Intermediate control

High intermediate control
Resistant control

Selection and parametrization of the biotest | **Comparison of distribution between repetitions** | **Study of the S/R of varieties**

Decisions — Susceptibility / resistance level of varieties

5 possibilities:

The choice depends on:

- the couple species / Pathogen
- the indication in the official test protocol.

1) **No** control bound

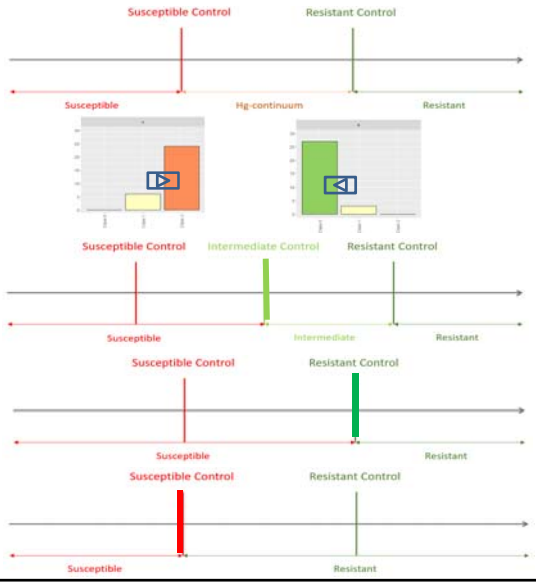
2) Class 1 mobile

3) IR control lower bound

4) **R** control lower bound

5) **S** control higher bound

Decisions rules



PATHOSTAT application - REPORTS

2 formats available with the **same content**, nevertheless:

.pdf format: quicker layout, more attractive, as a complete document	**.doc format:** slower page layout (additional image compression steps), easier possibilities to use parts of the report.

It includes:

- Minimum threshold number, *according the selected options*
- a summary of the Resistance test feature
- the results of the Comparison of distribution between replications (histogram)
- Homogeneity tests
  - regardless the variety
  - for each variety
- Dendrogram associated to the Study of the Susceptibility / Resistance of varieties
- Decisions
  - Cochran-Armitage results for each varieties
  - *According the chosen decision rule*, **proposal of conclusion**

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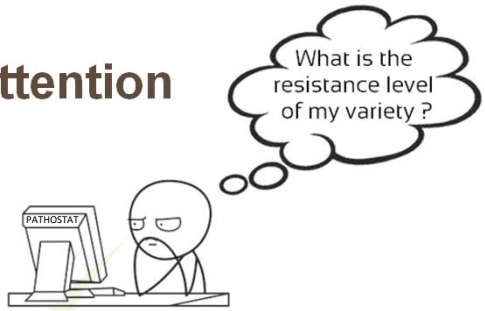
Any Question ?

Thank your for your attention



Now, it is up to !

https://www.geves.fr/tools/pathostat

In case of additional questions, you can contact:

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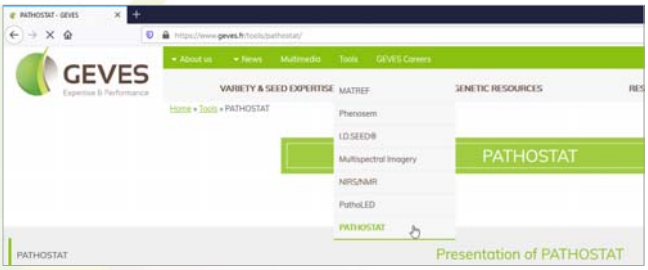

[Annex II follows]

ANNEX II

crea
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Centro di ricerca
orticoltura e florovivavismo

Disease resistance tests on *Solanum sisymbrifolium*, *S. torvum* and *S aethiopicum*: tomato and eggplant rootstoks - Italian laboratory experience

May, 11-15 2020

e-TWV, Brasilia, Brazil

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Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Tomato and eggplant rootstocks

Symptoms of *V. dahliae* on eggplant in a natural infected field

Soil-borne pathogens are among the most important pathogens of tomato and eggplant worldwide.

In tomato, stable characters of resistance to *Fusarium oxysporum* f. sp. *lycopersici* races and *Verticillium dahliae* are introduced in the commercial varieties. Recently, even resistance to *Pyrenochaeta lycopersici* is present in some varieties. A different scenario is reported for resistance to Fusarium and Verticillium in eggplant or in old tomato varieties and ecotypes.

As a consequence of that, and for the new legislation on the use of pesticides, the grafting on resistant rootstocks is becoming more and more used for Solanum species.

Resistance tests developed on tomato and eggplant rootstocks

Breeders are working on resistances to *Fusarium oxysporum* f. sp. *lycopersici* races, *V. dahliae*, *P. lycopersici* and *F. o.* f. sp. *melongenae*. Our laboratory made experience on the couple host/pathogen reported in the table (in green)

Species/hybrid (interspecific cross)	*Fusarium oxysporum* f. sp. *lycopersici* race 0	*Fusarium oxysporum* f. sp. *lycopersici* race 1	*Verticillium dahliae*	*Pyrenochaeta lycopersici*	*Fusarium oxysporum* f. sp. *melongenae*
TOMATO ROOTSTOCK					
S. sisymbrifolium	■	■	■	■	
S. lycopersicum x S. hirsutum	■	■			
EGGPLANT ROOTSTOCK					
Solanum torvum			■		■
Solanum aethiopicum			■	■	■
S. melongena × S. aethiopicum			■	■	■

3

Application of CPVO TP294/1 REV.3

Good experience with the application of **TP294/1 REV.3** to test resistance to *Fusarium oxysporum* f. sp. *lycopersici* races, *V. dahliae* and *P. lycopersici*

Resistance of a rootstock of *S. sisymbrifolium* to Fol 1

4

Application of CPVO TP294/1 REV.3

V. dahliae / eggplant rootstocks

Accession of *S. aethiopicum* susceptible to *V. dahliae*

Eggplant variety Cima Viola used as susceptible control

Accession of *S. torvum resistant* to *V. dahliae*

5

Resistance to *F. oxysporum* f. sp. *melongenae* on *S. torvum* e *S. aethiopicum*

F. o. f. sp. *melongenae* / eggplant rootstocks

Set-up of internal protocol based on the method of immersion of the roots in conidial suspension

10 days post inoculation

Susceptible control Cima Viola

23 days post inoculation

6

Application of CPVO TP294/1 REV.3

Resistance of a rootstock of *S. sisymbrifolium* to *P. lycopersici*

Symptoms on Marmande

Criticity in application of the draft protocol reported in the partial revision (2019)

7

Conclusion

All the tests, in their practical application, showed that the validated technical protocol for tomato rootstocks can be used even with these species.

Critical points that will be the object of further experiments for the application of the method to inoculate *P. lycopersici*

Set up of an internal protocol for resistance tests to Fusarium oxysporum f. sp. melongenae in eggplant and in eggplant rootstocks

Thank you for your attention!

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[End of Annex II and of document]