CPVO
Community Plant Variety Office

Bremia **: comparison of substrates**

Vegetable Variety Open Day

Harmonisation of resistance tests for DUS testing "Harmores"

Edinburgh (UK), 12 September 2017

Introduction

- Disease and pest resistance characteristics becoming increasingly important in vegetable DUS testing – however, no harmonised methodologies amongst examination offices nor breeders!

- Problem identified by main stakeholders, so concerted effort made to establish R&D project to tackle issue in a concerted manner

- CPVO co-funds projects aiming to harmonise at EU level, resistance tests of important vegetable disease and pests in CPVO protocols according to urgency of situation
  - **Projects coordinated by GEVES (FR)**

- To date, three R&D have been established in this area:
  - ✓ **Harmores 1 (French bean, tomato): 2004-2007**
  - ✓ **Harmores 2 (lettuce, pea, pepper): 2012-2015**
  - ✓ **Harmores 3 (melon, pea, tomato): 2016-2019**

Introduction

- CPVO co-funded three year project (2012-2015), coordinated by GEVES
- 7 examination offices (CZ, DE, ES, FR, GB, HU, NL) and 5 European Seed Association (ESA) members involved
- **Aims**: Harmonise at the European Union level, resistance tests to seven vegetable diseases:
    - *Bremia lactucae* in lettuce
    - *Fusarium oxysporum* f. sp. *pisi* race 1 in pea
    - *Ascochyta pisi* race C in pea
    - TMV: 0 in pepper,
    - PMMoV: 1.2 in pepper,
    - PMMoV:1.2.3 in pepper,
    - PVY: 0 in pepper

Why harmonise DUS resistance test protocols

- Better coherence of results between countries
- Better declarations from breeders and official tests
- Better definition and exchange of reference material

Methodology

- ✓ updated bibliography on the selected host/pathogen combinations
- ✓ available reference isolates with maintainer laboratories
- ✓ available reference varieties as resistant and susceptible controls
- ✓ optimised culture conditions for all the studied pathogens
- ✓ optimised test conditions
- ✓ harmonised techniques to be proposed to CPVO for implementation

Time frame and deliverables of project

Phase 1

- *Description and comparison of the existing tests*

Phase 2

- *Selection of common reference material*

Phase 3

- *Harmonisation and validation of resistance testing techniques*

Phase 4

- *Implementation of harmonised techniques into corresponding CPVO test protocols and UPOV Test Guidelines*

Collaboration and « hands on » approach

- Numerous exchanges during the project's duration
- Annual meetings between the project partners
- Practical workshops to harmonise interpretation of results

RESISTANT					
	No sporulation + no necrosis	No sporulation + necrosis	Weak sporulation (much less than susceptible control) + necrosis	Weak sporulation less than susceptible control not evolving between second and third notation + necrosis	In some cases very sparse sporulation can occur (without necrosis) and does not evolve between 2nd and 3rd notation
SUSCEPTIBLE			OTHER CASE	Normal sporulation (same level as susceptible control) with necrosis ➔ **in this case another test on bigger plants or other substrate must be undertaken**	
	Reduced sporulation (compared to susceptible control) without necrosis	Normal sporulation without necrosis			

Results and implementation

- Presentation of new notation scale to IBEB, and scientific posters at Eucarpia congresses 2015-16
- Updated robust test techniques proposed 2016 to CPVO
- CPVO implemented improved techniques in March 2017 via partial revisions to lettuce, pepper and pea protocols
- **Final step**: implementation by UPOV of improved techniques into:
  - ✓ **Revision of the Test Guidelines for lettuce TG/13/11** (approval at TC/53)
  - ✓ **Partial revision of the Test Guidelines for pepper TG/76/8 Rev.** (discussion at TWV/51)
  - ✓ **Partial revison of the Test Guidelines for pea TG/7/10 Rev.** (discussion at TWV/51)

Ad. 47: Resistance to Tobamovirus

1. Pathogen ..... *Tobacco mosaic virus* and *Pepper mild mottle virus*
2. Quarantine status ..... no
3. Host species ..... Sweet pepper, hot pepper, paprika and chilli – *Capsicum annuum* L.
4. Source of inoculum ..... GEVES[1] (FR), Naktuinbouw[2] (NL) or INIA[3] (SP)
5. Isolate ..... *Tobacco mosaic virus* race 0 (TMV: 0) strain Vi-6
Pepper mild mottle virus race 1.2 (PMMoV: 1.2) strain nt203
Pepper mild mottle virus race 1.2.3 (PMMoV: 1.2.3) strain Eve
The test protocols have been validated in a CPVO co-funded project[4] with these 3 isolates/races.
6. Establishment isolate identity ..... genetically defined pepper differentials (reference ISF site: http://www.worldseed.org/isf/differential_hosts.html)

	Pathotype	P0	P1	P1-2	P1-2-3	P1-2-3-4
	Code	TMV: 0 ToMV: 0 TMGMV: 0 BPMoV: 0	TMV: 1 TMGMV: 1 PaMMV: 1	PMMoV: 1.2	PMMoV: 1.2.3	PMMoV: 1.2.3.4
Variety	Gene					
Lamu, Early Calwonder	-	S	S	S	S	S
Trisana, Yolo Wonder	*L1*	R	S	S	S	S
Tabasco	*L2*	R	R	S	S	S
Solario F1, Novi3, PI159236	*L3*	R	R	R	S	S
Tom4, PI260429	*L4*	R	R	R	R	S

S= susceptible; R= resistant; TMV= *Tobacco mosaic virus*; ToMV= *Tomato mosaic virus*; PMMoV= *Pepper mild mottle virus*; TMGMV= *Tobacco mild green mosaic virus*; BPMoV= *Bell pepper mottle virus*; PaMMV= *Paprika mild mottle virus*

7. Establishment pathogenicity ..... Test on susceptible plants
8. Multiplication inoculum
8.1 Multiplication medium ..... Regeneration of the virus of plant material before inoculum preparation.
8.2 Multiplication variety ..... On susceptible pepper variety, Tobamovirus races may be multiplied on varieties which are selective for each particular race. For TMV, because tomato and tobacco *Nicotiana tabacum* cv. Samsun have large leaves and can produce a lot of inoculum, they are recommended for the multiplication of TMV: 0.
8.3 Plant stage at inoculation ..... see 10.3
8.4 Inoculation medium ..... see 10.1
8.5 Inoculation method ..... see 10.4
8.6 Harvest of inoculum ..... Symptomatic fresh leaves
8.7 Check of harvested inoculum ..... option: on young leaves of Nicotiana tabacum "Xanthi", check for local lesions after 5-7 days at 20-25°C
8.8 Shelf life/viability inoculum ..... fresh > 1 day in fridge, desiccated or juice > 1 year in fridge
9. Format of the test
9.1 Number of plants per genotype ..... At least 20 plants.
9.2 Number of replicates ..... -
9.3 Control varieties ..... TMV: 0:
Susceptible controls: Pepita or Lamu or Piquillo
Resistant controls: Fehérozon or Yolo Wonder
PMMoV: 1.2:
Susceptible controls: Fehérozön or Yolo Wonder or Lamu

[1] marref@geves.fr
[2] resistentie@naktuinbouw.nl
[3] zardoba@inia.es
[4] Harmores 2 CPVO project (http://www.cpvo.europa.eu/main/en/home/documents-and-publications/technical-projects-reports)

Ad. 37: Resistance to downy mildew (*Bremia lactucae*)

1. Pathogen ..... *Bremia lactucae*
2. Quarantine status ..... no
3. Host species ..... Lettuce - *Lactuca sativa* L.
4. Source of inoculum ..... GEVES[1] (FR), Naktuinbouw[2] (NL) or INIA (SP)[3]
5. Isolate ..... Bl: 20-27, 29-31 (the test protocol has been validated in a European CPVO co-funded project[4]).
6. Establishment isolate identity ..... (see point 9.3)
7. Establishment pathogenicity ..... Test on susceptible varieties
8. Multiplication inoculum
8.1 Multiplication medium ..... Lettuce plantlets
8.2 Multiplication variety ..... *Bremia* races may be maintained on varieties or breeding lines which are more or less selective for each particular isolate. It is essential to multiply Bl: 27 on selective plant material, e.g. NunDm17.
8.3 Plant stage at inoculation ..... see 10.3
8.4 Inoculation medium ..... Tap water
8.5 Inoculation method ..... see 10.4
8.6 Harvest of inoculum ..... see 10.1
8.7 Check of harvested inoculum ..... see 10.2
8.8 Shelf life/viability inoculum ..... 2 hours at room temperature; 2 days in the fridge
9. Format of the test
9.1 Number of plants per genotype ..... At least 20 separate plants of each variety should be tested to establish the uniformity.
9.2 Number of replicates ..... -
9.3 Control varieties ..... The host differential set that can distinguish all important *Bremia* races should always be used in tests, as a check on the identity of the isolate (see http://www.worldseed.org/isf/bob.html)
9.4 Test design ..... -
9.5 Test facility ..... Climate room
9.6 Temperature ..... 15°C-18°C
9.7 Light ..... Adequate for good plant growth. Seedling may have well opened cotyledons (fully or not yet fully expanded) but must not be not etiolate.
9.8 Season ..... -
9.9 Special measures ..... Plants may grow on wet blotting paper with or without a nutrient solution, on sand or on potting soil (see point 13). High humidity (>90%) is essential for infection and sporulation.
10. Inoculation
10.1 Preparation inoculum ..... Washing off from leaves by vigorous shaking in a closed container
10.2 Quantification inoculum ..... Counting spores; spore density should be 3.10^1-1.10^4
10.3 Plant stage at inoculation ..... Cotyledon stage
10.4 Inoculation method ..... Spraying till run-off. Option: reduced light 24 hours after inoculation.
10.5 First observation ..... Beginning of sporulation on susceptible varieties (around 7 days after inoculation)
10.6 Second observation ..... 3-4 days after first observation (around 10 days after inoculation)
10.7 Final observations ..... 14 days after inoculation. Two of these three observations may be sufficient; the third notation is optional for observation of

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Thank you for your attention!

Any questions ?