UPOV

International Union for the Protection of New Varieties of Plants

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Technical Committee **TC/55/23**

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PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO ROOTSTOCKS

Document prepared by experts from the Netherlands

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1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Tomato rootstocks (document TG/294/1 Corr. Rev. 2).

2. The TWV, at its fifty-third session, held in Seoul, Republic of Korea, from May 20 to 24, 2019, considered a proposal for a partial revision of the Test Guidelines for Tomato Rootstocks on the basis of documents TG/294/1 Corr. Rev. 2 and TWV/53/7 "Partial revision of the Test Guidelines for Tomato Rootstocks" and proposed the following changes (see document TWV/53/14 Rev. "Revised Report", paragraph 95):

(a) To change the denomination of the races of Characteristics 24.1, 24.2 and 24.3 "Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)";
(b) To change the explanation Ad. 24 in Chapter 8.2 "Explanations for individual characteristics";
(c) To change the example varieties of Characteristic 28 "Resistance to *Pyrenochaeta lycopersici* (Pl)";
(d) To change the explanation Ad. 28 in Chapter 8.2 "Explanations for individual characteristics"

3. The proposed changes are presented from page 2 in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

4. The TWV further agreed that the e-mail addresses below for obtaining the inoculum in all disease resistance explanations throughout the Test Guidelines for Tomato Rootstocks should be updated in the final adopted version of this partial revision of the Test Guidelines for Tomato Rootstocks as follows (see document TWV/53/14 Rev. "Revised Report", paragraph 96):

Geves: matref@geves.fr
Naktuinbouw: resistentie@naktuinbouw.nl
INIA: resistencias@inia.sp

Proposal to change the denomination of the races of Characteristics 24.1, 24.2 and 24.3 “Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)”

Current wording

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
24. **(+)**		**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Résistance à *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Resistenz gegen *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Resistencia a *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**		
24.1 **(*)**	**VG**	**– Race 0 (ex 1)**	**– Pathotype 0 (ex 1)**	**– Pathotyp 0 (ex 1)**	**– Raza 0 (ex 1)**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
24.2 **(*)**	**VG**	**– Race 1 (ex 2)**	**– Pathotype 1 (ex 2)**	**– Pathotyp 1 (ex 2)**	**– Raza 1 (ex 2)**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
24.3 **(*)**	**VG**	**– Race 2 (ex 3)**	**– Pathotype 2 (ex 3)**	**– Pathotyp 2 (ex 3)**	**– Raza 2 (ex 3)**		
QL		absent	absente	fehlend	ausente	Emperador	1
		present	présente	vorhanden	presente	Colosus	9

Proposed new wording

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
24. **(+)**		**Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Résistance à *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Resistenz gegen *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**	**Resistencia a *Fusarium oxysporum* f. sp. *lycopersici* (Fol)**		
24.1 **(*)**	**VG**	**– Race ~~0 (ex 1)~~ 0EU/1US**	**– Pathotype ~~0 (ex 1)~~ 0EU/1US**	**– Pathotyp ~~0 (ex 1)~~ 0EU/1US**	**– Raza ~~0 (ex 1)~~ 0EU/1US**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
24.2 **(*)**	**VG**	**– Race ~~1 (ex 2)~~ 1EU/2US**	**– Pathotype ~~1 (ex 2)~~ 1EU/2US**	**– Pathotyp ~~1 (ex 2)~~ 1EU/2US**	**– Raza ~~1 (ex 2)~~ 1EU/2US**		
QL		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Emperador	9
24.3 **(*)**	**VG**	**– Race ~~2 (ex 3)~~ 2EU/3US**	**– Pathotype ~~2 (ex 3)~~ 2EU/3US**	**– Pathotyp ~~2 (ex 3)~~ 2EU/3US**	**– Raza ~~2 (ex 3)~~ 2EU/3US**		
QL		absent	absente	fehlend	ausente	Emperador	1
		present	présente	vorhanden	presente	Colosus	9

Proposal to change the explanation Ad. 24 in Chapter 8.2 “Explanations for individual characteristics”

Current wording

Ad. 24: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)

1. Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
3. Host species.....	*Solanum lycopersicum*
4. Source of inoculum.....	Naktuinbouw[1] (NL) and GEVES[2] (FR)
5. Isolate.....	Race 0 (ex 1) (e.g. strains Orange 71 or PRI 20698 or Fol 071 1 (ex 2) (e.g. strains 4152 or PRI40698 or RAF 70 and 2 (ex 3) Individual strains may vary in pathogenicity
6. Establishment isolate identity	use differential varieties (see 9.3)
7. Establishment pathogenicity.....	on susceptible tomato varieties
8. Multiplication inoculum	
8.1 Multiplication medium.....	Potato Dextrose Agar, Medium “S” of Messiaen
8.4 Inoculation medium……...	water for scraping agar plates or Czapek-Dox culture medium (7 d-old aerated culture)
8.6 Harvest of inoculum.....	filter through double muslin cloth
8.7 Check of harvested inoculum	spore count; adjust to 10^6 per ml
8.8 Shelf-life/viability inoculum	4-8 h, keep cool to prevent spore germination
9. Format of the test	
9.1 Number of plants per genotype	at least 20 plants
9.2 Number of replicates……	1 replicate
9.3 Control varieties for the test with race 0 (ex 1)	
Susceptible……(*Solanum lycopersicum*) Marmande, Marmande verte, Resal	
Resistant for race 0 only……(*Solanum lycopersicum*) Marporum, Larissa, “Marporum x Marmande verte”, Marsol, Anabel	
Resistant for race 0 and 1 ……..(*Solanum lycopersicum*) Motelle, Gourmet, Mohawk	
Control varieties for the test with race 1 (ex 2)	
Susceptible ……(*Solanum lycopersicum*) Marmande verte, Cherry Belle, Roma	
Resistant for race 0 only ……..(*Solanum lycopersicum*) Marporum, Ranco	
Resistant for race 0 and 1 ……..(*Solanum lycopersicum*) Tradiro, Odisea	
Remark: ..... …….Ranco is slightly less resistant than Tradiro	
Control varieties for the test with race 2 (ex 3)	
Susceptible for race 2……Emperador	
Resistant for race 0, 1 and 2……Colosus	
9.4 Test design……	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5 Test facility……	glasshouse or climate room
9.6 Temperature…….....	24-28°C (severe test, with mild isolate) 20-24°C (mild test, with severe isolate)
9.7 Light……	12 hours per day or longer
9.8 Season ……	all seasons
9.9 Special measures……	slightly acidic peat soil is optimal; keep soil humid but avoid water stress
10. Inoculation	
10.1 Preparation inoculums……	aerated Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or scraping of plates
10.2 Quantification inoculums……	spore count, adjust to 10^6 spores per ml, Lower concentration for a very aggressive isolate
10.3 Plant stage at inoculation……	10-18 d, cotyledon to first leaf
10.4 Inoculation method……	roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option
10.7 Final observations……	14-21 days after inoculation
11. Observations	
11.1 Method ……	visual
11.2 Observation scale……	Symptoms: growth retardation, wilting, yellowing, vessel browning extending above cotyledon
11.3 Validation of test……	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties	
absent ……[1]	severe symptoms
present……[9]	mild or no symptoms

13. Critical control points:
Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature. Standards near borderline R/S will help to compare between labs.

[1] Naktuinbouw: resistentie@naktuinbouw.nl

[2] GEVES; Valerie.GRIMAULT@geves.fr

Proposed new wording

Ad. 24: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)

1.	Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	Naktuinbouw[3] (NL), GEVES[4] (FR) or INIA[5] (ES)
5.	Isolate	race ~~0 (ex 1)~~0EU/1US (e.g. strains Orange 71 or PRI 20698 or Fol 071) race ~~1 (ex 2)~~1EU/2US (e.g. strains 4152 or PRI40698 or RAF 70) race ~~2 (ex 3)~~2EU/3US (e.g. strain Fol029) ~~individual strains may vary in pathogenicity~~
6.	Establishment isolate identity	use differential varieties (see 9.3)
7.	Establishment pathogenicity	on susceptible tomato varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	Potato Dextrose Agar, Medium “S” of Messiaen
8.4	Inoculation medium	water for scraping agar plates or Czapek-Dox culture medium (7 d-old aerated culture)
8.6	Harvest of inoculum	filter through double muslin cloth
8.7	Check of harvested inoculum	spore count; adjust to 10^6 per ml
8.8	Shelflife/viability inoculum	4-8 h, keep cool to prevent spore germination
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	1 replicate
9.3.1	Control varieties for the test with race ~~0 (ex 1)~~ 0EU/1US	
	Susceptible	(*Solanum lycopersicum*) Marmande, Marmande verte, Resal
	Resistant ~~for race 0 only~~	Emperador, Colosus and (*Solanum lycopersicum*) “Marporum x Marmande verte”, ~~Marsol, Anabel~~ Motelle, Gourmet, Mohawk, Ranco, Tradiro
	~~Resistant for race 0 and 1~~	~~(*Solanum lycopersicum*) Motelle, Gourmet, Mohawk~~
9.3.2	Control varieties for the test with race ~~1 (ex 2)~~1EU/2US	
	Susceptible	(*Solanum lycopersicum*) Marmande verte, Cherry Belle, Roma, Marporum, Ranco
	~~Resistant for race 0 only~~	~~(*Solanum lycopersicum*) Marporum, Ranco~~
	Resistant ~~for race 0 and 1~~	Emperador, Colosus and (*Solanum lycopersicum*) Tradiro, Odisea, “Motelle x Marmande verte”
9.3.3	Control varieties for the test with race ~~2 (ex 3)~~2EU/3US	
	Susceptible ~~for race 2~~	Emperador and (*Solanum lycopersicum*) Marmande verte, Motelle, Marporum
	Resistant ~~for race 0, 1 and 2~~	Colosus and (*Solanum lycopersicum*) Tributes, Murdoch, “Marmande verte x Florida”
9.4	Test design	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5	Test facility	glasshouse or climate room
9.6	Temperature	24-28°C (severe test, with mild isolate) 20-24°C (mild test, with severe isolate)
9.7	Light	12 hours per day or longer
9.8	Season	all seasons
9.9	Special measures	slightly acidic peat soil is optimal; keep soil humid but avoid water stress

[3] Naktuinbouw: resistentie@naktuinbouw.nl

[4] GEVES: matref@geves.fr

[5] INIA: resistencias@inia.sp

10.	Inoculation	
10.1	Preparation inoculum	aerated Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or scraping of plates
10.2	Quantification inoculum	spore count, adjust to 10^6 spores per ml, lower concentration for a very aggressive isolate
10.3	Plant stage at inoculation	10-18 d, cotyledon to first leaf
10.4	Inoculation method	roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option
10.7	Final observations	14-21 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	symptoms: growth retardation, wilting, yellowing, vessel browning extending above cotyledon
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.
12.	Interpretation of test results in comparison with control varieties	
	absent ..... [1]	severe symptoms
	present ..... [9]	mild or no symptoms
13.	Critical control points	Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature.

Proposal to change the example varieties of Characteristic 28 “Resistance to *Pyrenochaeta lycopersici* (Pl)”

Current wording

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
28. **(+)**	**VG**	**Resistance to *Pyrenochaeta lycopersici* (Pl)**	**Résistance au *Pyrenochaeta lycopersici* (Pl)**	**Resistenz gegen *Pyrenochaeta lycopersici* (Pl)**	**Resistencia a *Pyrenochaeta lycopersici* (Pl)**		
QL		absent	absente	fehlend	Ausente	Zaralto	1
		present	présente	vorhanden	Presente	Emperador	9

Proposed new wording

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
28. **(+)**	**VG**	**Resistance to *Pyrenochaeta lycopersici* (Pl)**	**Résistance au *Pyrenochaeta lycopersici* (Pl)**	**Resistenz gegen *Pyrenochaeta lycopersici* (Pl)**	**Resistencia a *Pyrenochaeta lycopersici* (Pl)**		
QL		absent	absente	fehlend	ausente	~~Zaralto~~	1
		present	présente	vorhanden	presente	Emperador	9

Proposal to change the explanation Ad. 28 in Chapter 8.2 “Explanations for individual characteristics”

Current wording

Ad. 28: Resistance to *Pyrenochaeta lycopersici* (Pl)

1. Pathogen .....*Pyrenochaeta lycopersici*
3. Host species .....*Solanum lycopersicum*
4. Source of inoculum .....-
5. Isolate .....-
7. Establishment pathogenicity.....biotest
8. Multiplication inoculum
8.1 Multiplication medium .....V8 Agar
8.2 Multiplication variety .....susceptible tomato variety
8.3 Plant stage at inoculation .....seed
8.4 Inoculation medium.....mixture of soil, e.g. (70%), sand (20%) and inoculum (10.1) (10%)
or soil mixed with diseased roots cut to small pieces
8.5 Inoculation method .....sowing, or transplanting at fruit maturity
8.6 Harvest of inoculum .....diseased roots are harvested after 2-4 months
8.7 Check of harvested inoculum .....visual inspection of lesions on roots
8.8 Shelf-life/viability inoculum .....the fungus will not die quickly, but may lose its pathogenicity within a week after isolation on an agar medium
9. Format of the test
9.1 Number of plants per genotype .....20 plants
9.2 Number of replicates…… 1 replicate
9.3 Control varieties.....
susceptible:.....Zaralto and (*Solanum lycopersicum*) Montfavet H 63.5
resistant: .....Emperador and (*Solanum lycopersicum*) Kyndia, Moboglan, Pyrella
9.5 Test facility.....greenhouse or climate cell
9.6 Temperature .....day 24°C, night 14°C
9.7 Light .....12 h minimum
10. Inoculation
10.1 Preparation inoculum.....e.g. double-autoclaved mixture of soil with 10% oatmeal added
e.g. Incubate for 10-14 d at 20°C with occasional, repeated turning
10.3 Plant stage at inoculation .....6 weeks
10.4 Inoculation method .....transplanting into mixture of soil, sand and inoculum (8.4)
or soil mixed with diseased roots cut to small pieces
or naturally infected soil
10.7 Final observations.....6-8 weeks after transplanting (flowering plant)
11. Observations
11.1 Method.....visual
11.2 Observation scale .....Symptoms: brown lesions on roots
11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties

absent	……	[1]	symptoms
present	……	[9]	no symptoms

13. Critical control points:
The fungus loses its pathogenicity quickly after isolation on an agar medium. It is advisable to keep the isolate alive on living plants.

Proposed new wording

Ad. 28: Resistance to *Pyrenochaeta lycopersici* (Pl)

1.	Pathogen	*Pyrenochaeta lycopersici*
2.	Quarantine status	No
3.	Host species	*Solanum lycopersicum*
4.	Source of inoculum	GEVES[6] (FR)
5.	Isolate	e.g. strain Pl 21
6.	Establishment isolate identity	On susceptible plant
8.	Multiplication inoculum	
8.1	Multiplication medium	Messiaen agar or synthetic medium
8.4	Inoculation medium	Autoclaved grains (e.g. barley)
8.5	Inoculation method	Mix of contaminated grains (e.g. 1 kg) with inoculum (e.g. medium from 2 Petri dishes with mycelium)
8.6	Harvest of inoculum	After 3 weeks
9.	Format of the test	
9.1	Number of plants per genotype	At least 20
9.2	Number of replicates	1 replicate
9.3	Control varieties	Susceptible : (*Solanum lycopersicum*) Marmande verte Resistant : Emperador and (*Solanum lycopersicum*) Garance
9.4	Test design	Add non inoculated plants
9.5	Test facility	Greenhouse or climatic chamber
9.6	Temperature	20°C
9.7	Light	At least 12h
10.	Inoculation	
10.1	Preparation inoculum	Homogenize the contaminated grains
10.2	Quantification inoculum	-
10.3	Plant stage at inoculation	3-4 leaf stage
10.4	Inoculation method	Transplanting of plantlets in a mixture of soil (e.g. 3750 ml of soil with 750 ml of inoculum)
10.7	Final observations	40 days post inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	Class 0: no necrosic lesion on roots Class 1: few small and uncoloured necrotic lesions Class 2: some brown necrotic lesions clearly visible (less than half the surface of the pivot) Class 3: several brown necrotic lesions clearly visible (more than half the surface of the pivot) Class 4: complete necrosis or destruction of the pivot
11.3	Validation of test	Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12.	Interpretation of data in terms of UPOV characteristic states	Any variety judged to be of the same resistance level or higher than Garance is judged as resistant. Classes 0, 1 and 2 are commonly judged as resistant – Note 9 Classes 3 and 4 are commonly judged as susceptible – Note 1

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[6] GEVES: matref@geves.fr